BIOASSESSMENT OF CONTAMINATED SEDIMENTS WITH SPECIAL REFERENCE TO IMPACT ON THE MICROORGANISMS OF THE RECEIVING WATER.

FINAL REPORT TO ONTARIO MINISTRY OF THE ENVIRONMENT

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Abstract

Two bioassays have been developed for a rapid assessment of the potential impact of sediment dredging and disposal on (a) phytoplankton photosynthesis, and, (b) microbial phosphate uptake in lakes. Photosynthesis is measured in the presence of sediments using the ^{14}C method at a series of light intensities. The ^{14}C-labelled photosynthate is extracted with DMSO. The effects of sediment on microbial phosphate uptake are assessed using $^{32}P\text{-}PO_4$. These direct bioassay results are compared with the effects of elutriates of sediments, prepared by the standard EPA method.

Sediments from Toronto Harbour, added to lakewater at 2% $^{v}/v$, depressed Pmax, the light-saturated rate of photosynthesis, by up to 80%. In contrast, elutriates, added at equivalent dosages, resulted in a 20% depression, at most. Sediments and elutriates also had very different effects on microbial phosphate uptake: depending on the season, sediments acted either as a source or a sink of phosphate, but elutriates always acted as a source of phosphate for microorganisms.

The depression of photosynthesis by Toronto Harbour sediments correlates with the high concentrations of heavy metals and CB in the sediments. On the basis of bioassays with phosphorus-sufficient and deficient cultures of algae, we attribute the less drastic effects of elutriates on photosynthesis to heavy metal - phosphorus binding in the medium and/or inside the cells. In growth bioassays with algal cultures, the algae introduced with the sediment consistently outcompeted the diatom, and often the blue-green algae as well, but the green algae generally remained dominant. We predict that

dredging, and dredged sediment disposal operations, would result in drastic changes of the phytoplankton species composition in lakewater, particularly in the spring.

Résumé

Nous avons mis au point deux essais biologiques visant à évaluer rapidement les effets éventuels, dans les lacs, du dragage et de l'élimination des sédiments sur a) la photosynthèse du phytoplancton et b) l'assimilation de phosphate par les micro-organismes. La photosynthèse se mesure en présence de sédiments en utilisant du ^{14}C à diverses intensités de saturation lumineuse. Le photosynthétat marqué au ^{14}C est extrait à l'aide de diméthylsulfoxyde. Quant aux effets des sédiments sur l'assimilation de phosphate, ils sont évalués au moyen de $^{32}P\text{-}PO_4$. Ces résultats immédiats sont comparés aux effets des sédiments élutriés suivant la méthode en usage à l'Agence pour la protection de l'environnement (EPA).

Les sédiments du port de Toronto, ajoutés à de l'eau de lac à 2 % v/v, ont réduit la Pmax (photosynthèse maximale) jusqu'à 80 %. Par contre, les élutriés, ajoutés à des doses équivalentes, l'ont tout au plus diminuée de 20 %. Sédiments et élutriés avaient aussi des effets très différents sur l'assimilation de phosphate par les mirco-organismes; selon la saison, les sédiments agissaient soit comme une source, soit comme un réceptacle de phosphate, mais les élutriés en fournisssaient toujours.

Si les sédiments du port de Toronto réduisent la photosynthèse, il faut dire que leur concentration élevée de métaux lourds et de chlorobenzènes y est pour quelque chose. D'après les essais biologiques sur des cultures d'algues dont certaines présentaient une carence en phosphore alors que les autres en contenaient suffisamment, nous attribuons les effets moindres des élutriés sur la photosynthèse à la liaison qui se produit entre les métaux lourds et le phosphore dans le milieu ou dans les cellules. Au cours d'essais biologiques sur la croissance, les algues introduites avec les sédiments l'ont emporté chaque fois sur les diatomées, et souvent même sur les algues bleu-vert, tandis que les vertes étaient généralement dominantes. Selon nous, le dragage et l'élimination des sédiments modifieraient beaucoup la composition du phytoplancton dans nos lacs, notamment au printemps.

Introduction

Biosassays at the primary producer level can detect subtle changes in the normal functioning of aquatic systems in response to contaminant load. In combination with data on bioaccumulation, such bioassays can act as early warning devices for detection of potentially irreversible changes in upper trophic levels. In a normal aquatic ecosystem bacteria play a vital and major role primarily as decomposers of organic material. As an integral part of their metabolic processes bacteria recycle inorganic nutrients essential to the primary producers. Of these phosphorus is of prime concern in aquatic ecosystems since it is the nutrient which controls the growth of phytoplankton, the major primary producers in lakes. In spite of the basic dependence of primary producers on normal bacterial functioning, bioassays which assess bacterial metabolic responses to contaminants are rare to non-existent.

In bioassays aimed at detecting the effects of sediment on primary producers the presence of the sediment leads to technical difficulties. This may be the reason why sediment elutriates have been used instead of sediments. Sediment elutriation procedures were developed by the US Corps of Engineers (USEPA, 1977 in Munawar et al. 1983) following evidence that bulk chemical characterisations, which measure total concentrations of contaminants in sediments, are unsatisfactory for evaluation of the actual contaminant loading to an ecosystem during dredged sediment disposal. Unfortunately, several researchers began using elutriates in bioassays without any tests of the assumption that the elutriates simulate adequately the effects of whole sediment on biota, (e.g. Plumb 1975, Flint and Lorefice, 1978,

Munawar et al 1983, Mayfield and Munawar 1983). Recent comparisons of the effects of elutriates and of whole sediments on phosphorus kinetics in lakewater showed that elutriate addition invariably increased phosphorus concentration but addition of sediment did not have a predictable outcome (Nalewajko 1985, Ewing 1987). The P-state of the microbial populations in the receiving water was important in setting the direction of net P flux, to, or from, the sediments. Further, the effects of elutriates on phosphorus availability and on photosynthesis showed a significant negative correlation (C. Nalewajko and Ewing 1987; Ewing 1987). In other words, elutriates that have high concentrations of phosphate may be not toxic to phytoplankton in spite of elevated concentrations of metals, but the actual sediments, which generally did not increase phosphorus concentrations of lakewater by much, if at all, potentially could be very toxic. These data make the use of elutriates in any bioassay suspect.

Another shortcoming of the elutriates is that most of the bacteria present in the sediments are excluded from the elutriate during the preparation procedures. The bacteria in sediments exceed lakewater populations by about 10^3, on a volume basis, and most likely play a major role on phosphorus and other nutrient and contaminant uptake kinetics during dredging and dredged-sediment disposal operations. Using lake column simulators Smith et al (1977) reported a dramatic increase in short term ^{14}C-acetate mineralization rates following the addition of dredged spoils. Also, phosphate was taken up faster, both by abiotic and biological processes, in response to dredged spoil addition.

Smith et al., 1977 also measured the effect of dredged spoil addition on in situ photosynthesis. Depressions of 25% and 54% at loadings of 3.3 and 33 g $.day^{-1}$ were attributed to decreased light intensity for photosynthesis. The possibility of comcomitant toxicity of the dredged spoils to the primary producers was not considered. Because of this dual effect of dredged spoils it is necessary to keep one factor (e.g. light) constant while investigating the other (e.g. toxicity).

Objectives:

The overall objective was to develop simple but sensitive bioassays for the assessment, and prediction of the impact of sediment disposal on open water primary producers and bacteria. Specifically, the objective was to assess the effect of the sediment in question on (a) phosphorus availability to the open water microorgnisms (phytoplankton plus bacteria;) (b) bacterial growth rates as assessed by DNA synthesis, and (c) phytoplankton growth rates as assessed by the Pmax (light-saturated photosynthesis) to chlorophyll a ratio (assimilation number).

Methods and Materials

Sampling Site:

Sediment was collected from a site in Toronto Harbour (Stn 1354) which is known to be heavily contaminated with a range of metals and organic toxins (Persaud et al., 1985). Samples were collected from a launch provided by the Ontario Ministry of the Environment. Lakewater samples for the phytoplankton bioassay were collected about 2 km offshore, from a station South-West of the Toronto Islands. Sampling was carried out on June 8 and November 6-7 1986, and on July 28, 1987.

Collection of sediment and lakewater:

Sediments were collected using a Ponar Grab sampler, and stored at 5^{o} C in the dark in sealed polyethylene bags. Bioassays with lakewater were carried out within a few hours of sample collection but algal culture bioassays were done with the stored sediments. Lakewater samples were collected from about 0.5 m depth directly into a polycarbonate carboy. Bioassays were started within about 2 h of collection of the water.

Elutriate Preparation:

Elutriates for use in phytoplankton bioassays were prepared using the method of Mudroch & Davies (1985) from freshly obtained sediments. Sediments were elutriated with 0.2µm filtered lakewater. In bioassays with algal cultures PO_4-free sterile CHU 10 was used instead of lakewater. Elutriates were made fresh on the day of experimentation.

Cultures and Culture Media:

The cyanobacterium Anabaena flos-aquae (Lyng.) Breb. was obtained from the Utex Culture Collection (Utex #1444). The green alga Ankistrodesmus falcatus (Corda) Ralfs and the diatom Diatoma elongatum (Lyngb.) Ag. were isolated from a sample of Lake Ontario water using the single cell fishing technique, and grown in CHU 10 medium (Nichols, 1973) with a soil extract added at 1% (v/v). All media used were sterilized by autoclaving before use. The 3 species were maintained at 22^{o} C, and 18^{o} C respectively. Once the cultures had grown and become dense they were inoculated into CHU 10 with no soil extract.

Phosphorus sufficient culture was obtained by inoculating the algae into 18 litres of CHU 10 medium and incubating at 6.0×10^{15} quanta cm^{-2} s^{-1}. This culture was used when the density reached approximately 1.0×10^{5} cells/ml. Phosphorus deficient cultures were obtained by inoculating P-sufficient cells into 18 litres of P-free CHU 10. This culture was diluted at approximately weekly intervals with PO_4-free CHU 10, and a trace metal solution and the vitamins biotin, thiamine, and B12 (Morel et al., 1979) were added to maintain the health of the cultures. This was continued until the ^{32}P-PO_4 turnover time was less than 20 minutes.

Cultures of Pseudomonas fluorescens were grown on 3% yeast extract in CHU 10 medium at 20° C.

Phytoplankton and Bacterial Enumeration:

Phytoplankton:- A 250 ml aliquot of each lakewater sample was preserved with 1% Lugol's iodine solution and stored at 5° C. The samples were later settled in counting chambers and phytoplankton was enumerated using the Utermohl technique (Lund et al., 1958) on a Wild M40 inverted microscope. Enough cells were counted to obtain a standard error of about 10%. Species identifications were based on Prescott (1978) and Findlay & Kling (1979). Biovolumes were estimated by measurement of cells and approximation to geometric figures. Biomass was calculated from biovolume assuming a specific gravity of 1.00.

Bacteria:- A 50 ml aliquot of each lakewater sample was preserved for bacterial enumeration with a 3% formalin solution and stored at 5° C until enumeration. Bacteria were stained with 1% Acridine Orange for three minutes and filtered onto a 0.2 m pore size black Sartorius filter (Daley & Hobbie, 1975). Enumeration was carried out in a Leitz Dialux microscope fitted with a Ploepak 2.3 fluorescent vertical illuminator, HBO-50 high pressure measuring lamp, and BG38, K480, and 2 x KP490 filters. Replicate fields were counted until a standard error of 10% was achieved. Population density in cultures of Pseudomonas fluorescens was measured as above.

Chlorophyll Analysis:

Triplicate 500 ml or 1 L aliquots of lakewater were filtered onto GF/C filters and frozen in glassine envelopes until extraction.

Chlorophylls were extracted using the DMSO extraction technique of Shoaf & Lium (1976), as modified by Burnison (1980). Absorbance was measured at 664, 647 and 630 nm on a Cary model 118 double beam spectrophotometer. Chlorophyll concentrations were calculated using the modified equation of Jeffery and Humphrey (1975),

$$\text{Chl a } (\mu g\ mL^{-1}) = (11.85E_{664} - 1.54E_{647} - 0.08E_{630}).$$

Phosphorus Chemistry:

Phosphorus was measured both as isobutanol extractable phosphorus (PO_4), and as total phosphorus (TP). Concentrations in the samples were calculated by linear regression of a standard curve. Standards were made with $K_2\ HPO_4$ in deionized distilled water and were made fresh for each set of analyses. All glassware used in phosphorus analysis was previously soaked for 24 hours in 2% H_2SO_4 and rinsed several times in distilled water.

Extractable Phosphate:- Isobutanol extractable phosphate was analyzed using the molybdenum blue method of Chamberlain & Shapiro (1969). Extraction was conducted onboard ship until the stable molybdophosphoric acid was obtained. This extract was stored at 5° C and reduced with stanous chloride before measurement. Absorbance was measured at 698 nm on a Cary model 118 spectrophotometer.

Total Phosphorus:- For total phosphorus analysis, lakewater samples were digested using the autoclave/potassium persulfate method of Menzel and Corwin (1965). This method is unsuitable for samples with high concentrations of particulate matter (Jefferies et al., 1979) so a more rigorous sulfuric-nitric acid digestion (American

Public Health Assoc., 1965) was used for elutriate and sediment samples. Digested samples were then analysed using the ascorbic acid combined reagent (AACR) method of Murphy and Riley (1962). Absorbance was measured at 698 nm, the point of maximal absorbance of the reduced molybdenum blue complex, in the wavelengths available on the Cary spectrophotometer.

Trace Metal Analyses:

Samples were collected in 250 mL polyethylene bottles, acidified to pH 2 with concentrated HNO_3, and stored in the dark at 5^{o} C until analysed. Analysis of trace metals in the elutriates and lakewater samples was done using flameless atomic absorbtion spectroscopy. All analyses were done by Dr. A. Mudroch at C.C.I.W. in Burlington, Ontario.

PCB Analysis:

PCB in sediments and elutriates were analyzed courtesy of Dr. A. Mudroch. Samples for organic analysis were Soxhlet extracted prior to analysis by capillary, electron capture detector, gas chromatographic analyses. (Oliver and Bourbonniere 1985).

Phosphorus Kinetics:

The uptake of PO_4 was followed in time-course experiments using carrier-free $^{32}P\text{-}PO_4$ (New England Nuclear Ltd) as a tracer (Lean & Nalewajko, 1976). Lakewater or culture was incubated in the dark in 250 ml glass bottles. At intervals, over 1.5 hours, six aliquots were filtered onto 0.2 m pore size polycarbonate membrane filters (Nuclepore corp.), PCS scintillation fluor was added and radioactivity of filters measured in a Beckman model LS-6800 Scintillation counter.

Radiophosphorus kinetics in lakewater containing sediments were examined by following the disappearance of $^{32}P\text{-}PO_4$ from the solution compartment over a 2 h period. Aliquots of filtrate were assayed for radioactivity after addition of PCS fluor. The initial slope of the ln % radioactivity remaining in the filtrate vs time curve is the rate constant, k, of ^{32}P uptake. The reciprocal, $^1/_k$, is the turnover time of the external phosphate pool.

Photosynthesis vs. Irradiance:

Photosynthesis was measured using the ^{14}C method (Vollenweider, 1969). Lakewater or algal culture (250 ml) with and without sediment were incubated in six 400 ml polycarbonate bottles. Elutriates were added at 10% (v/v) dosages, while sediment was added at 2% (v/v) as this represented the amount of sediment used in a 10% (v/v) elutriate dosage. In all experiments where elutriates and sediments were added, the control series was diluted to the same extent using 0.2 m filtered lakewater (FLW). P-free, sterile CHU 10 was used to dilute the control series. 200 l of 20 ci.mL $^{14}C\text{-}NaHCO_3$ (Amersham) were added to each bottle and the bottles arranged in series in the P-I box, which is a constant temperature water bath illuminated from one end with a 500W quartz halogen lamp (Philips 12013R). Irradiance was measured inside each of the six bottles with a Model QSL-100 quantum meter (Biospherical Instruments Inc.) and ranged from less than 3.0×10^{14} to approximately 8×10^{16} quanta $cm^{-2}s^{-1}$. All bottles in the P-I box were agitated at approximately 40 minute intervals. In bottles with sediment, the irradiance was measured at frequent intervals during the agitation cycle. Irradiance was then plotted against time and the curve integrated using a Model 620000 planimeter (Keuffel &

Esser Co.) to yield the average irradiance in each bottle.

In the control series (lakewater or culture without sediment), 25 mL aliquots were filtered in triplicate on 0.45 m cellulose acetate membrane filters. The filters were rinsed with lakewater which had been pre-filtered through glass fiber (GF/C) filters (Whatman Ltd.), or with sterile Chu 10 medium, and dissolved in 15 mL of PCS scintillation fluor (Amersham) in plastic vials. The radioactivity of the filters was measured in a Beckman Model LS-6800 scintillation counter. The total amount of radioactivity added was measured in 1.0 mL aliquots to which 0.2 mL of phenethylamine had been added.

Both the control series and the experimental (plus sediment) series were also filtered in triplicate (50 mL) onto GF/C glass fiber filters (Whatman Ltd.) and then frozen for later analysis. These filters were later thawed and ground using a Model RZR1-64 stirrer (Caframo Ltd.) with a teflon grinding bit. The ^{14}C labelled photosynthate was extracted from the cells in 10 mL of dimethyl sulfoxide (DMSO) using the method of Shoaf and Lium (1976) modified by Burnison (1980). The DMSO was incubated at 65^{o}C in a Model B7005-2 water bath (Canlab Ltd.) for 30 minutes. DMSO extracts were passed through GF/C filters and the filtrate caught in plastic scintillation vials. To these vials 5 mL of PCS fluor was added and the radioactivity was measured as above. Quench correction curves were prepared with DMSO chlorophyll and the coloured compounds extracted from the sediments by DMSO.

For calculation of assimilation numbers, Pmax from P-I curves was converted to units of µg C using the dissolved inorganic carbon concentration (DIC) calculated from total alkalinity values for Lake Ontario reported by Allen et al., (1969). In experiments with cultures the value for DIC in Chu 10 (8.87 (mg L^{-1}) was used. The assimilation number for each series was calculated as Pmax/Chla.

Following the completion of P-I curve experiments, aliquots from the control and the sediment-treated bottles were transferred into sterile flasks and kept at $20^{o}C$, 3 x 10^{16} quanta $cm^{-2}.sec^{-1}$. Subsamples were taken every 2-3 days for enumerations of algal population densities.

DNA analyses

DNA was analysed by the method of Falkowski and Owens (1982). The basis of this method lies in the use of the fluorescent dye, DAPI, (4, 6 - diamidino - 2^{1} - phenylindole 2 HCl) which binds quantitatively and specifically to DNA. Instead of a spectrofluorometer (which was not available) we used a Turner fluorometer fitted with a 360 nm excitation filter and a 455 nm emission filter.

Statistical Analysis:

The photosynthesis data were compared with a pairwise Student's t-Test using the points that made up the light saturated part of the P-I curve (Pmax).

Rate constants were calculated using linear regression analysis.

Results

^{32}P Kinetics

The effects of elutriates (10% $^{v}/_{v}$) and whole sediments (2% $^{v}/_{v}$) on ^{32}P uptake kinetics in lakewater were compared on June 8, 1986. (Fig. 1A). The addition of elutriate caused a large and statistically significant increase in phosphate turnover time, but addition of whole sediment initially resulted in a very rapid uptake of ^{32}P, then a return to levels similar to the control.

On subsequent dates (Nov. 6-7, 1986 and July 28, 1987) only the effects of (2% $^{v}/_{v}$) sediment addition on ^{32}P kinetics were investigated [Fig. 1B and 1C]. Phosphate turnover time in lakewater was slowest (42.7 h) in November 1986, followed by June 1986 (3.0 h) and July 1987 (0.75 h). The initial rapid uptake of ^{32}P was not observed on addition of sediment.

DNA in bacteria

DNA concentrations in cultures of Pseudomonas fluorescens amounted to 3% on a volume basis. No significant changes occurred on exposure for 4 h to 10% $^{v}/_{v}$ elutriate (June 8, 1986).

It proved impossible to extract DNA quantitatively in the presence of 2% $^{v}/_{v}$ sediment. Recovery of known amounts of DNA was erratic, suggesting that variable amounts of DNA were being contributed by the bacteria associated with the sediments. In view of this, further attempts to develop a DNA-based bacterial bioassay were abandoned.

Photosynthesis - irradiance curves

On June 8, the addition of elutriate at 10% ($^{v}/_{v}$) had no effect on the light-limited part of the P-I curve (Fig. 2) but depressed Pmax by 24% (P 0.05). In this experiment the radioactivity of filters containing ^{14}C- labelled phytoplankton was measured directly.

Fig. 3 shows results based on DMSO extraction of ^{14}-C labelled photosynthate at the end of the P-I experiment. In the control series, 60% of all ^{14}C- fixed by the phytoplankton was extracted by DMSO. Addition of elutriate depressed Pmax by about 30% (P 0.05) of the control, but whole sediment depressed it by 77%. Neither elutriate nor sediment had an effect on ^{14}C incorporation at irradiances sub-saturating for photosynthesis.

It was important to ascertain whether the depression observed with the addition of whole sediment was due to the fluctuating light caused by the settling of the sediment or to toxic effects of the sediment itself. The experimental set-up is illustrated in Fig. 4. Figure 5 shows the results of the series run with the phytoplankton inside the tube, i.e. physically separated from the sediment. The series with just lakewater outside the test-tubes showed a significant (P< 0.05) 18% depression of Pmax. The series with sediment outside the tube showed a 29% depression that was also significantly different from control, but not significantly different from the series with lakewater.

The effects of sediment on phytoplankton P-I curves were also investigated on Nov. 6-7, 1986 (Fig. 6) and July 28, 1987 (Fig. 7). In November Pmax was depressed by 19%, that is, depression was less

severe than in July: Depression in July 1987 was very similar to the June 1986 value.

For each date assimilation numbers were calculated as the ratio of Pmax to chlorophyll a (Table 1). Mean phytoplankton growth rates were estimated from assimilation numbers (Cote and Platt, 1983). In June 1986 and Nov. 1986 mean generation time ($^{1}/_{\mu}$) of the phytoplankton was 25 days, but growth was much faster in July 1987 (MGT = 6.6 days).

Bioassays with algal cultures: P-I curves and growth rates

Results from experiments on the effects of sediments (collected on 3 occasions: June 8 and Nov. 6-7, 1986; July 28, 1987) on P-I curves of P-deficient and P-sufficient cultures of the 3 bioassay species (see methods) are shown in figs. 8-13. For each species growth rates were calculated from assimilation numbers, and also measured directly (Table 2).

In all flasks to which sediment was added "seeding" with a variety of algae had taken place. After the 2-3 week growth period, algae introduced with the sediment consistently outcompeted the diatom, and often the blue-green alga as well, but the green alga remained dominant. A subjective ranking of abundance of these introduced species is given in Table 3.

Discussion

The dramatic difference in Pmax depression by sediments compared to sediment elutriates confirms our earlier concern (Ewing 1987) as well as the concern expressed by Cheam et al (1976), Grimwood and McGhee (1979) and Munawar et al (1983) about the use of elutriates in bioassays. It is clear that elutriate-based algal bioassays would drastically underestimate the potential impact of dredging and dredged sediment disposal on primary producers. In the past, sediment elutriates, rather than the actual sediments were used in bioassays because the researchers were unable to overcome logistic problems such as scintillation counting of ^{14}C in the presence of sediment (eg. Shuba et al 1977, Munawar et al 1983). In neither case were any comparisons attempted between responses to elutriates and to sediment. In spite of this, the latter authors continue to recommend the elutriate-based phytoplankton bioassays (IJC 1987).

Sediments from Stn 1354 in Toronto Harbour contain higher concentrations of phosphorus, lead and chromium than mid-lake Ontario and Niagara River sediments (Table 4) but concentrations of copper, zinc, mercury, arsenic and nickel are comparable as are PCB concentrations (Persaud et al 1985, Table 4 this report). The relatively low toxicity of elutriates to phytoplankton could be the result of more extensive metal-phosphate binding reactions in the elutriates than in the sediment. In contrast, the lipophilic organic contaminants are not likely to come into solution during elutriation but will be taken up by algal cells in contact with sediment particles. We suggest that these two processes are at the basis of the differential toxicity of elutriates and sediments to

phytoplankton.

Chlorinated hydrocarbons (PCB, DDT) have been shown to be toxic to many species of algae (Fisher et al 1976; MacFarlane et al 1972; Mosser et al 1972; O'Connors et al 1978). The latter authors demonstrated that PCB decreased ^{14}C photosynthetic uptake of phytoplankton, and affected the species composition within a few days as a result of greater sensitivity of diatoms. The final outcome was a change to predominance of smaller algae among the phytoplankton. The authors hypothesised that this change would have severe implications on the food web. In the Great Lakes, a concern about similar deleterious effects of PCB on phytoplankton were expressed by Kilham and Tiltman (1975).

The greater depression of Pmax by sediments in June and July compared to November suggests that phytoplankton species composition is an important factor to consider when selecting dates for sediment dredging and disposal operations. Although $^{32}PO_4$ turnover time was slower in November than in June or July the difference was probably not a significant factor in moderating sediment toxicity. Unlike the elutriates, which were less toxic to P-sufficient than to P-deficient algae, the effect of sediment was not related to the P-status of the cultures used in bioassays (Table 2).

The individuality of responses at the species level is reinforced by comparisons of Pmax depression by sediments among the 3 species of algae, as well as between the natural phytoplankton and the algal cultures. First, Pmax of the 3 bioassay species in culture was depressed by 90-99%, which is considerably greater than the 19-77% depression observed in lakewater. Second, Ankistrodesmus was less

depressed than Anabaena or Diatoma.

Growth rates of the bioassay species measured experimentally did not agree with the rates calculated from Assimiliation Numbers. Most likely, this is a result of the experimental design. The growth experiments were started with the supernatant of the ^{14}C P-I experiments, that is, the growth medium contained very little of the sediment. The presence of sediment had to be avoided to prevent light limitation during growth in the flasks, which were illuminated from below. Thus the sediment dosage in the growth experiments was much lower - about 1/100 - of the dosage in the P-I experiments. The lack of growth depression under these conditions is in line with our hypothesis that direct contact between the sediment and the algae is necessary for the full toxic effects of the sediment to be manifested. It also implies that during a dredging, or dredged sediment disposal operation, the detrimental effects are confined to the period of actual contact between the algae and the sediments, and decrease substantially as the sediments settle out.

Invasion by algae from the added sediments confounds the interpretation of the potential impact of dredging on phytoplankton communities. Concomitant with the introduction of toxic metals, organic contaminants, bacteria and nutrients, is the arrival of algae which had settled to the sediments. Within several days of the start of the growth bioassay, these introduced algae began to grow, and by 2-3 weeks, they collectively outnumbered the bioassay species.

Conclusions

It is clear from our work that elutriates do not model adequately the toxicity of sediments to phytoplankton. The reasons for the discrepancy are not elucidated as yet but we hypothesise that phosphate-metal chemistry and organic contaminant solubility are important contributing factors.

Our second conclusion is that photosynthetic response of phytoplankton to a dredging or dumping event may not be related to subsequent growth. Third, we conclude that the most serious effect of dredging and dredged sediment disposal is a change in phytoplankton community structure. Thus we appear to be in agreement with Schindler (1987) who concluded that community structure, rather than function, is a better indicator of pollution stress on an ecosystem.

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Table 1

Summary of Pmax, Chla, Assimilation Numbers (AN) and growth rates, natural phytoplankton samples before and after exposure to Toronto Harbour sediments, Stn 1354.

Date	Treatment	Pmax $\mu g.C.L^{-1}h^{-1}$	Chla $\mu g.L^{-1}$	AN	Growth rate μ, $div.^{-1}$
THI					
8 June 1986	Control	4.20	25.16	0.17	0.04
	+ sediment	0.96	25.16	0.04	0.01
TH2					
18 Nov. 1986	Control	1.37	9.16	0.15	0.04
	+ sediment	1.11	9.16	0.12	0.03
TH3					
28 July 1987	Control	5.62	10.41	0.54	0.15
	+ sediment	1.65	10.41	0.16	0.05

Table 2

Effects of Toronto Harbour sediments, Stn 1354 on Pmax, Chla, Assimilation Numbers (AN) and growth rates of P-sufficient and P-deficient algal cultures. $^{32}PO_4$-P turnover times in controls were as follows:

Anabaena : P-sufficient 658 min; P-deficient 13.8 min

Ankistrodesmus : P-sufficient 287 min; P-deficient 13.3 min

Diatoma : P-sufficient 357 min; P-deficient 19.4 min

NG - no growth

Culture	Pmax µg.C. $\cdot(10^9$ cells$)^{-1}.h^{-1}$ (min)	Chla µg. $\cdot 10^9$ cells$)^{-1}$	AN	Growth rate µ(div.d^{-1}) (measured)	Growth rate µ(div.d^{-1}) (calculated)
Anabaena P-sufficient					
Control	978.3	140.0	6.99	1.52	0.36
+TH 1 sediment 8 June/86	7.1	140.0	0.051	0.02	0.86
+ TH 2 sediment 18 Nov/86	5.6	140.0	0.040	0.015	0.90
+ TH 3 sediment 28 July/87	6.3	140.0	0.045	0.017	1.02
Ankistro-desmus P-sufficient					
Control	844.8	500.0	1.68	0.53	0.80
+TH 1 sediment 8 June/86	55.1	500.0	0.11	0.04	1.14
+TH 2 sediment 18 Nov/86	60.7	500.0	0.12	0.05	0.90
+TH 3 sediment 28 July/87	217.1	500.0	0.43	0.16	1.23

Table 2 (continued)

	Pmax µg.C. $(10^9 cells)^{-1}.h^{-1}$ (min)	Chla µg. $(10^9$ cells$)^{-1}$	AN	Growth rate µ(div.d)$^{-1}$ (measured)	Growth rate µ(div.d^{-1}) (calculated)
Diatoma P-sufficient					
Control	3657.4	1010.0	3.62	0.010	- NG -
+TH 1 sediment 8 June/86	59.5	1010.0	0.058	0.015	- NG -
+TH 2 sediment 18 Nov/86	84.5	1010.0	0.084	0.011	- NG -
+TH 3 sediment 28 July/87	178.3	1010.0	0.177	0.015	- NG -
Anabaena P-deficient					
Control	1344.5	250.0	5.38	1.29	- NG -
TH 1	6.9	250.0	0.028	0.011	- NG -
TH 2	7.8	250.0	0.032	0.012	- NG -
TH 3	3.8	250.0	0.015	0.016	- NG -
Ankistrodesmus P-deficient					
Control	156.2	470.0	0.33	0.123	- NG -
TH 1	16.9	470.0	0.035	0.014	- NG -
TH 2	11.7	470.0	0.025	0.009	- NG -
TH 3	13.6	470.0	0.029	0.011	- NG -
Diatoma P-deficient					
Control	1073.8	530.0	2.03	0.49	- NG -
TH 1	11.9	530.0	0.022	0.007	- NG -
TH 2	11.9	530.0	0.022	0.007	- NG -
TH 3	11.0	530.0	0.021	0.006	- NG -

Table 3

Summary of species invading algal bioassay cultures from Toronto Harbour sediments (combined data for experiments with sediments collected June 1986, November 1986 and July 1987).

INVADING SPECIES	ANABAENA		ANKISTRODESMUS		DIATOMA	
	P-Suff.	P-Def.	P-Suff.	P-Def.	P-Suff.	P-Def.
CYANOPHYCEAE:						
Anabaena spp.	1	1			2	
Lyngbya spp.		4				
Merismopedia sp.	4	4				
Oscillatoria spp.		4				4
CHLOROPHYCEAE:						
Ankistrodesmus falcatus	3	3	1	1	4	3
Chlamydomonas spp.	4	2	4	3	2	2
Chlorella spp.	3	2	4	4	2	3
Coelastrum sp.	4	4	4	4	4	4
Microspora sp.					2	
Oocystis spp.	3	3	4	4	3	3
Pediastrum boryanum					4	
Pediastrum spp.				4	3	3
Scenedesmus acutus	3	3	4	3	3	4
Scenedesmus quadricauda		4		4	1	
Scenedesmus spp.	3	2	4	3	3	3
Small green spp.	3	4	4	2	3	2
Staurastrum paradoxum				4	4	4
BACILLARIOPHYCEAE:						
Cocconeis sp.				4	4	
Cyclotella sp.					3	4
Cymbella sp.					4	
Diatoma spp.	3		3		4	1
Fragilaria spp.	4	3			4	3
Metosira spp.	3	3	3	2	3	3
Navicula spp.	3	3	3	4	3	4
Nitzschia spp.	3	3	4	2	1	3
Small unidentified diatoms	4	4				
Synedra spp.	3	3			3	3
Tabellaria fenestrata		3			1	3
Tabellaria flocculosa	3	3			2	3
CHRYSOPHYCEAE:						
Chromulina/Ochromonas spp.	3	3	5	5	4	3
Lagerheimia sp.					5	5
Small unidentified spp.	4	3	4	3	4	4
Final proportion of bioassay species, % total number of individuals.	25%	20%	50%	40%	10%	25%
No. of TAXA: 32						

Table 3 (continued)

SCALE:

1	DOMINANT SPP.
2	VERY COMMON SPP.
3	COMMON SPP.
4	RARE SPP.
5	VERY RARE SPP.

NOTE: The most recent sediment (July 1987) had a greater diversity of invading algal species than older sediments (e.g. November, 1986 and June 1986).

Table 4

Metals and organic contaminants in Toronto Harbour sediments, Stn. 1354 July, 1987; Lake Ontario Stn. 33A and Niagara River station 529.

Contaminant	Concentration µg/g		
	Stn.1354	Stn.33A	Stn.529
Fe	1.92	5.40	3.22
Mn	0.04	0.30	0.06
P	0.21	0.07	0.15
Cu	37	85	95
Zn	490	485	340
Pl	279	143	120
Col	5.7	0.9	1.3
Hg	0.31	0.49	0.68
As	15	37	23
Ni	75	103	80
Cr	135	89	83
PCB (total)	-	430	540
HCB	-	70	88
DDE	-	73	80
DDD	-	21	18
DDT	-	33	47

Figure legends

Fig. 1A to 1C $^{32}P\text{-}PO_4$ uptake kinetics in lakewater control -✱-; lakewater with 10% $^{v}/_{v}$ elutriate -△-; lakewater with 2% $^{v}/_{v}$ sediment -□-. 1A: Toronto Harbour, Stn. 1354, June 8, 1986; 1B: Nov. 18, 1986; 1C: July 28, 1987.

Fig. 2 Photosynthesis - irradiance (P-I) curve, lakewater control -✱-; lakewater with 10% $^{v}/_{v}$ elutriate -△-. Toronto Harbour, Stn. 1354, June 8, 1986.

Fig. 3 P-I curve based on DMSO extractable ^{14}C- photosynthate. Lakewater control -✱-; lakewater with 10% $^{v}/_{v}$ elutriate -△-; lakewater with 2% $^{v}/_{v}$ sediment -□-. Toronto Harbour, Stn. 1354, June 8, 1986.

Fig. 4 Incubation bottle used to determine the effect of fluctuating irradiance on photosynthesis.

Fig. 5 P-I curves of algae in polycarbonate bottle control -✱-; inside tube surrounded by culture medium -△-; inside tube surrounded by medium containing (2% $^{v}/_{v}$) sediment -□-.

Fig. 6 P-I curves based on DMSO - extractable ^{14}C- photosynthate. Lakewater control -□-; lakewater with 2% $^{v}/_{v}$ sediment -+-. November 18, 1986.

Fig. 7 P-I curves based on DMSO - extractable ^{14}C- photosynthate. Lakewater control -□-; lakewater with 2% $^{v}/_{v}$ sediment -+-. July 28, 1987.

Fig. 8A and B P-sufficient Anabaena culture: P-I curves based on DMSO-extractable ^{14}-C photosynthate. 8A Control; 8B plus 2% $^{v}/_{v}$ sediments Toronto Harbour, Stn. 1354, -□- June 8, 1986; -+-. Nov. 18, 1986; -◇- July 29, 1987. ($^{32}PO_4$-P turnover time, control: 658 mins.).

Fig. 9A and B As Fig. 8A and B but with P-deficient Anabaena. ($^{32}PO_4$-P turnover time, control : 13.8 mins.).

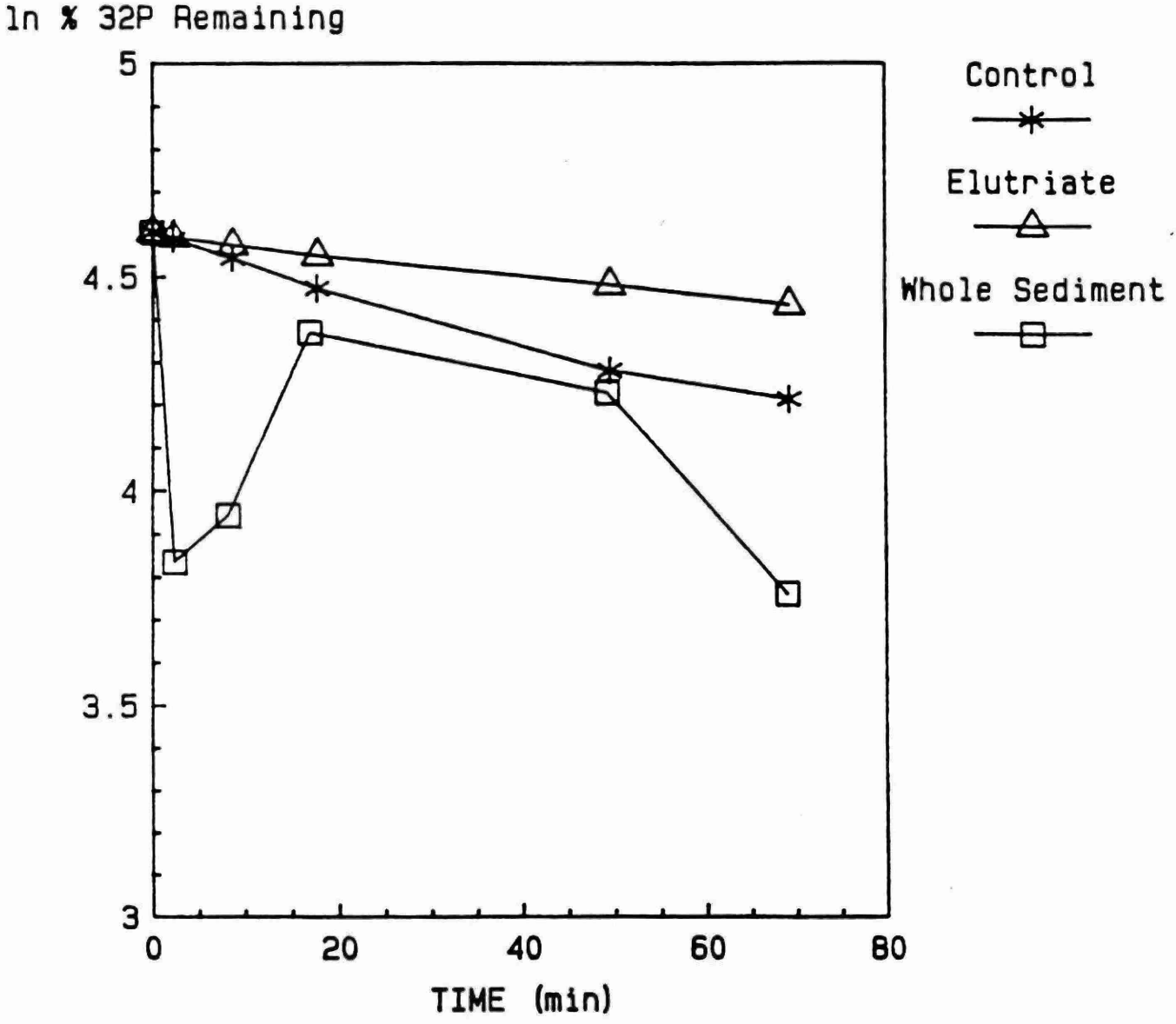
Fig. 10A and B P-sufficient Ankistrodesmus culture: P-I curves based on DMSO-extractable ^{14}C photosynthate. 10A Control; 10B plus 2% $^{v}/_{v}$ sediment, Toronto Harbour Stn. 1354. -□- June 8, 1986; -+- Nov. 18, 1986; -◇- July 28, 1987. ($^{32}PO_4$-P turnover time, control: 287 mins.).

Fig. 11A and B As Fig. 10A and B but with P-deficient Ankistrodesmus. ($^{32}PO_4$ turnover time, control: 13.3 mins.).

Fig. 12A and B P-sufficient Diatoma cultures: P-I curves based on DMSO-extractable ^{14}C photosynthate. 12A control; 12B plus 2% $^{v}/_{v}$ sediment, Toronto Harbour Stn. 1354. -□- June 8, 1986; -+- Nov. 18, 1986; -◇- July 28, 1987. ($^{32}PO_4$-P turnover time, control: 357 mins.).

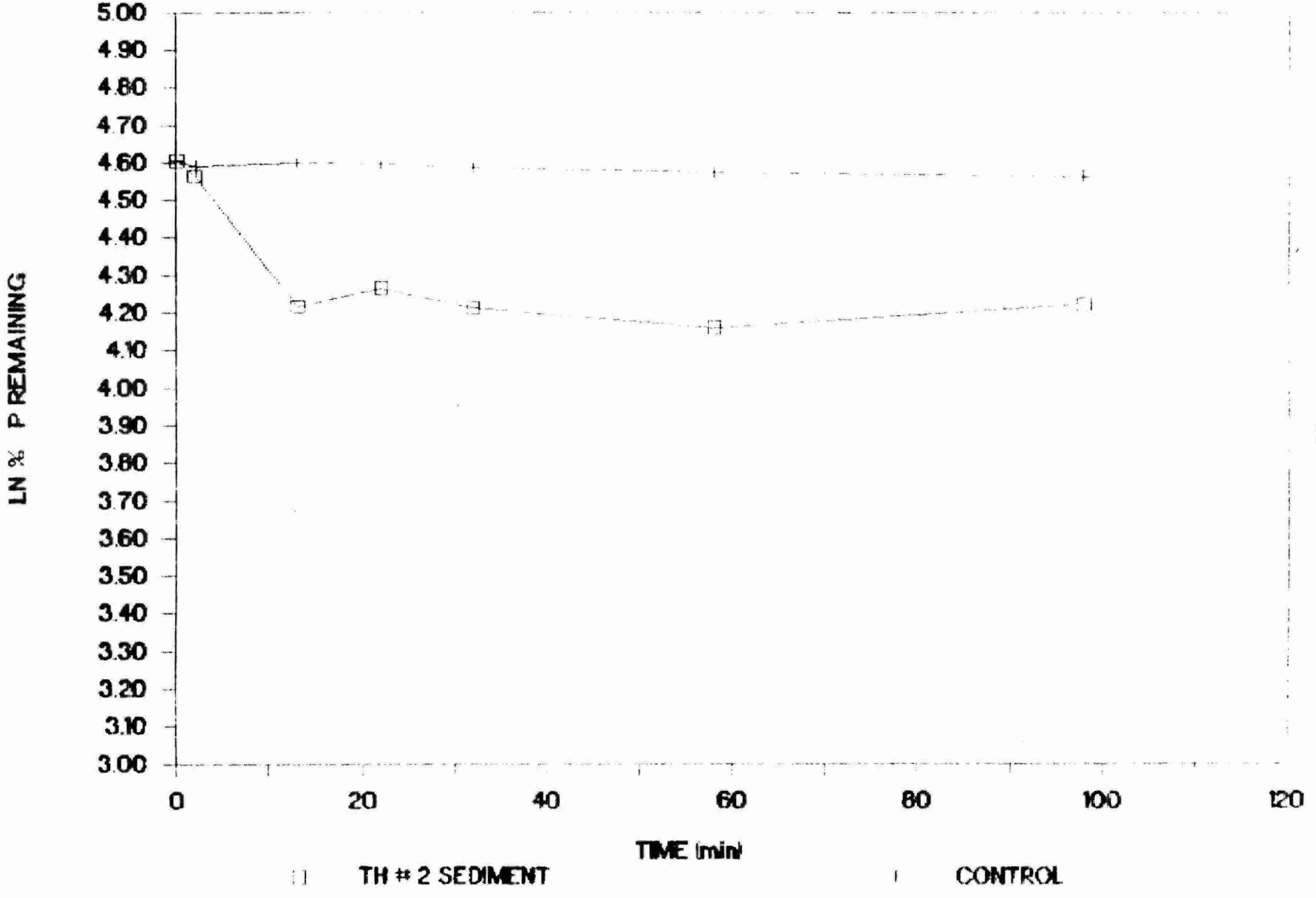
Fig. 13A and B As Fig. 12A and B but with P- deficient Diatoma. ($^{32}PO_4$-P turnover time, control: 19.4 mins.).

Fig 1A



Filtered on 0.2μm Nuclepore

Fig 13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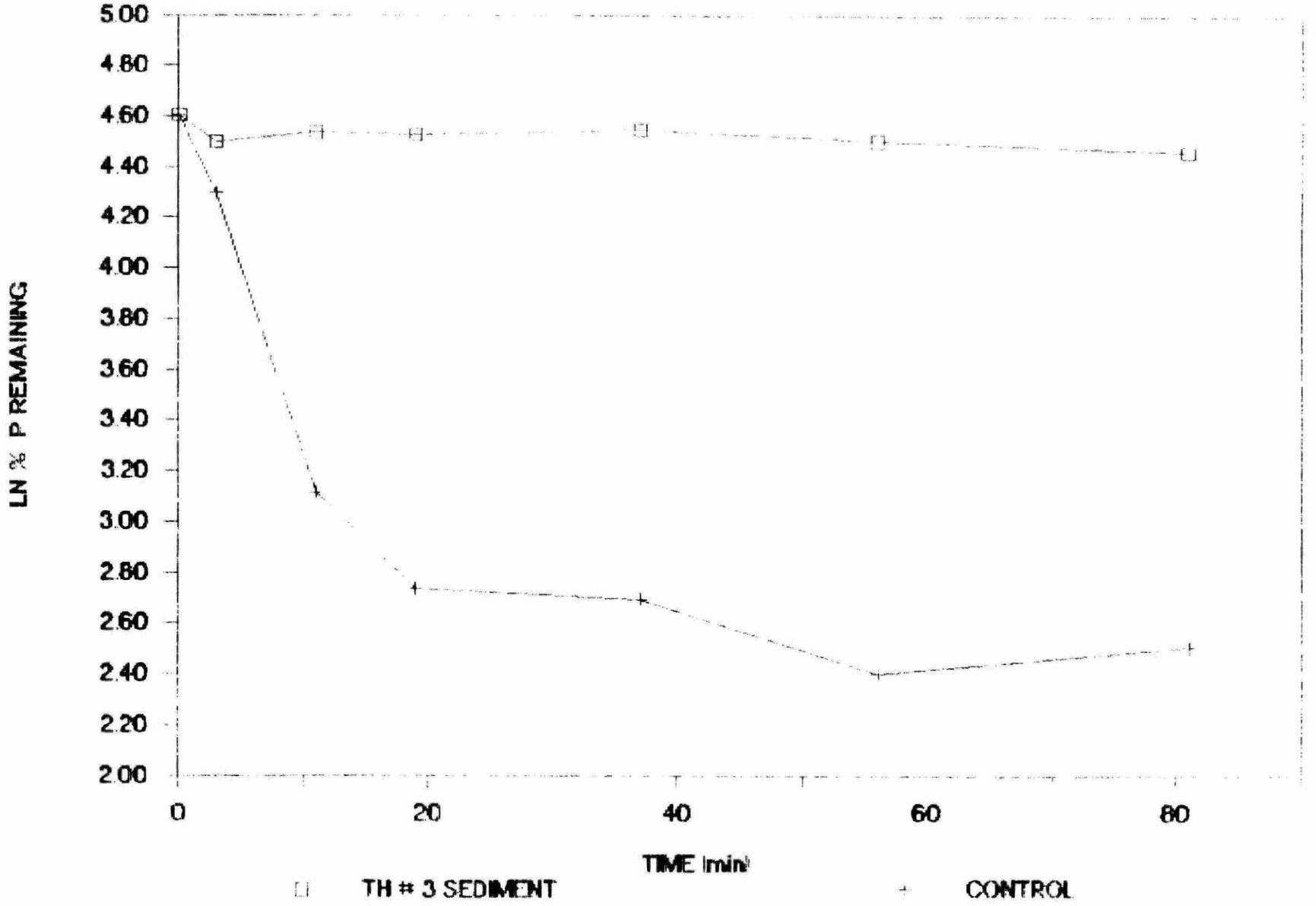
4.90
4.80
4.70
4.60
4.50
4.40
4.30
4.20
4.10
4.00
3.90
3.80
3.70
3.60
3.50
3.40
3.30
3.20
3.10
3.00
LN % P REMAINING
0
20
40
60
80
100
120
TIME (min)
TH # 2 SEDIMENT
CONTROL


LN % P REMAINING
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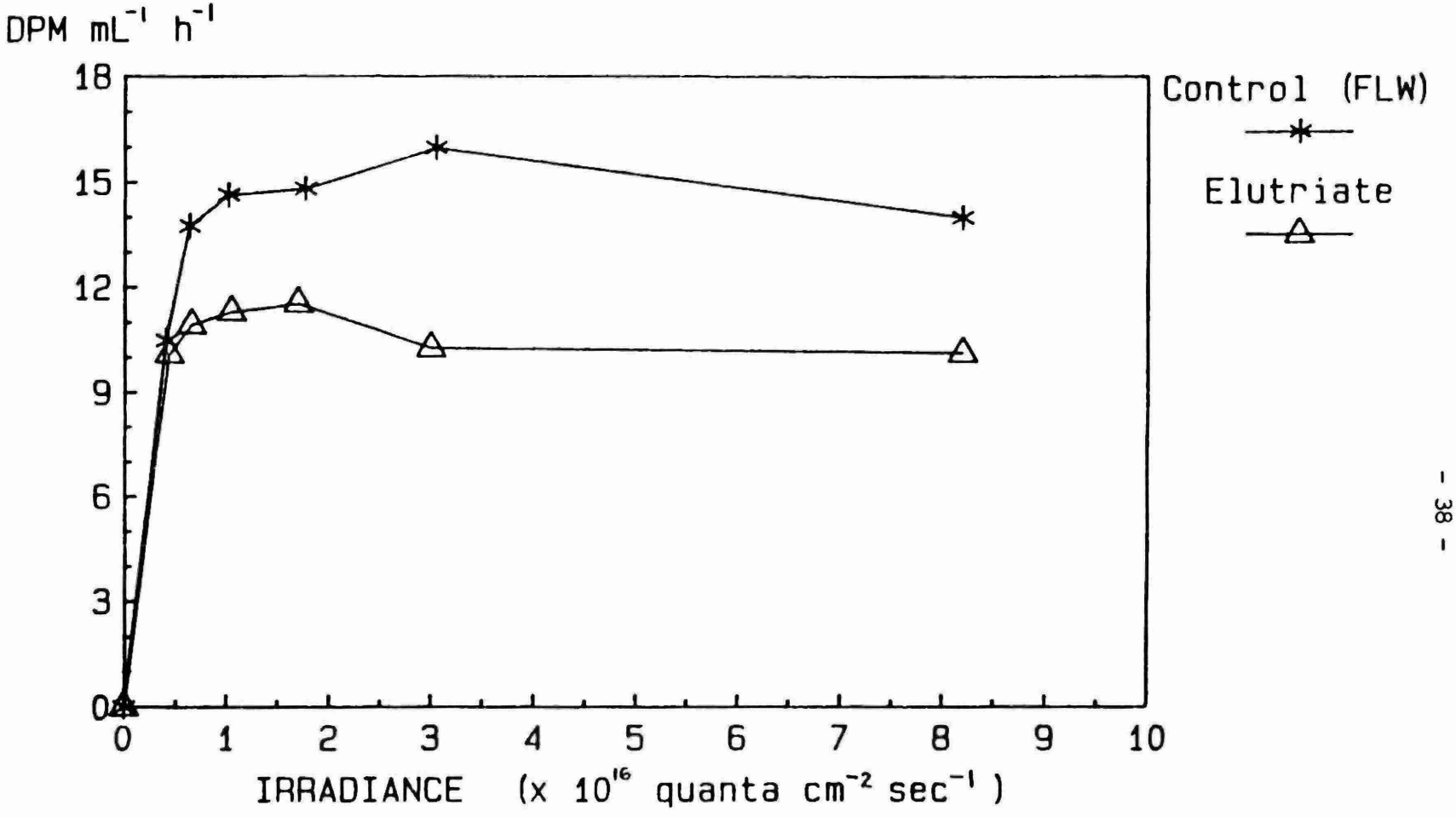
4.80
4.60
4.40
4.20
4.00
3.80
3.60
3.40
3.20
3.00
2.80
2.60
2.40
2.20
2.00
0
20
40
60
80
TIME (min)
TH # 3 SEDIMENT
CONTROL

Fig 2



Filtered on 0.45µm Sartorius

Fig 3

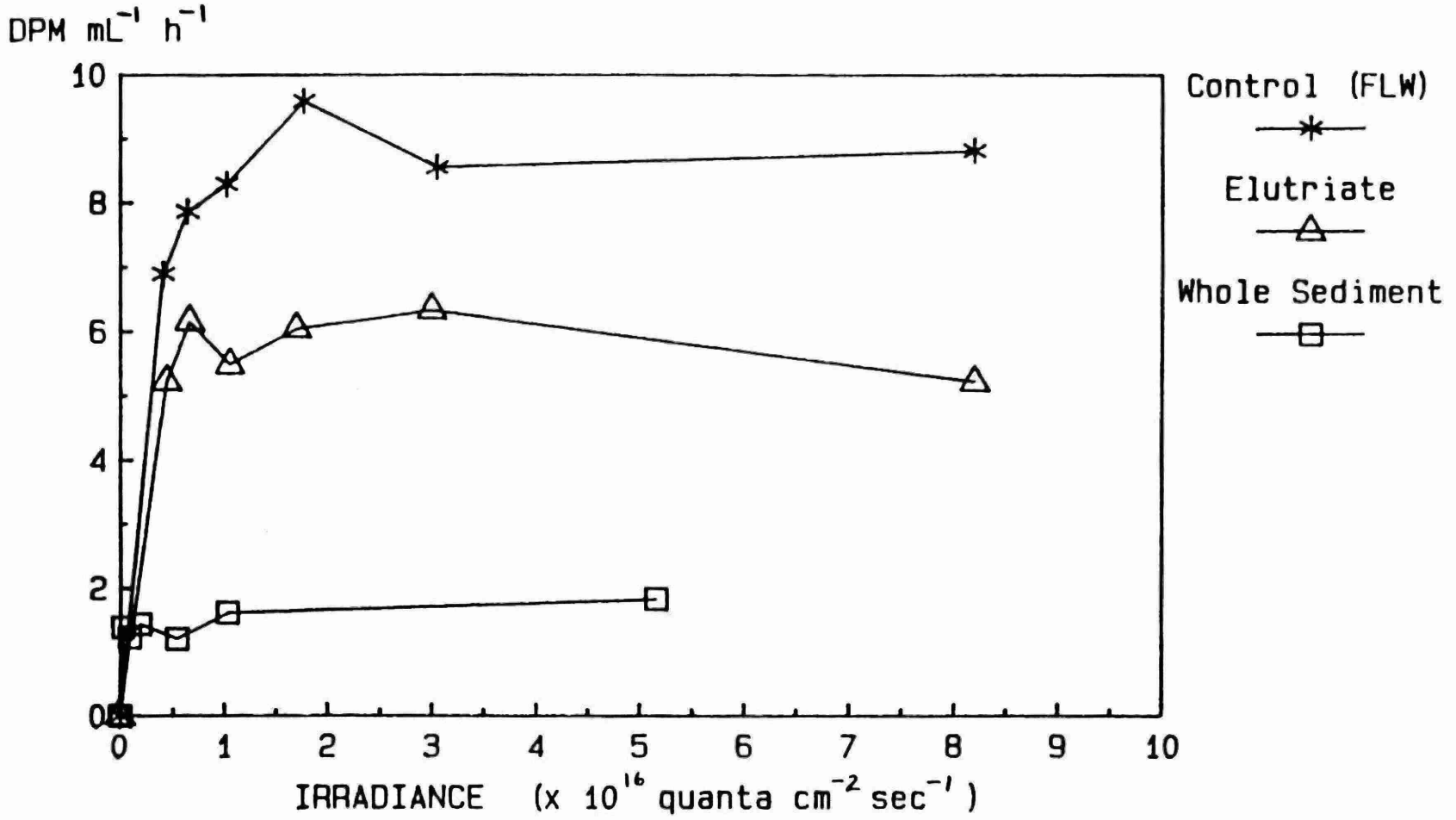


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Fig 4

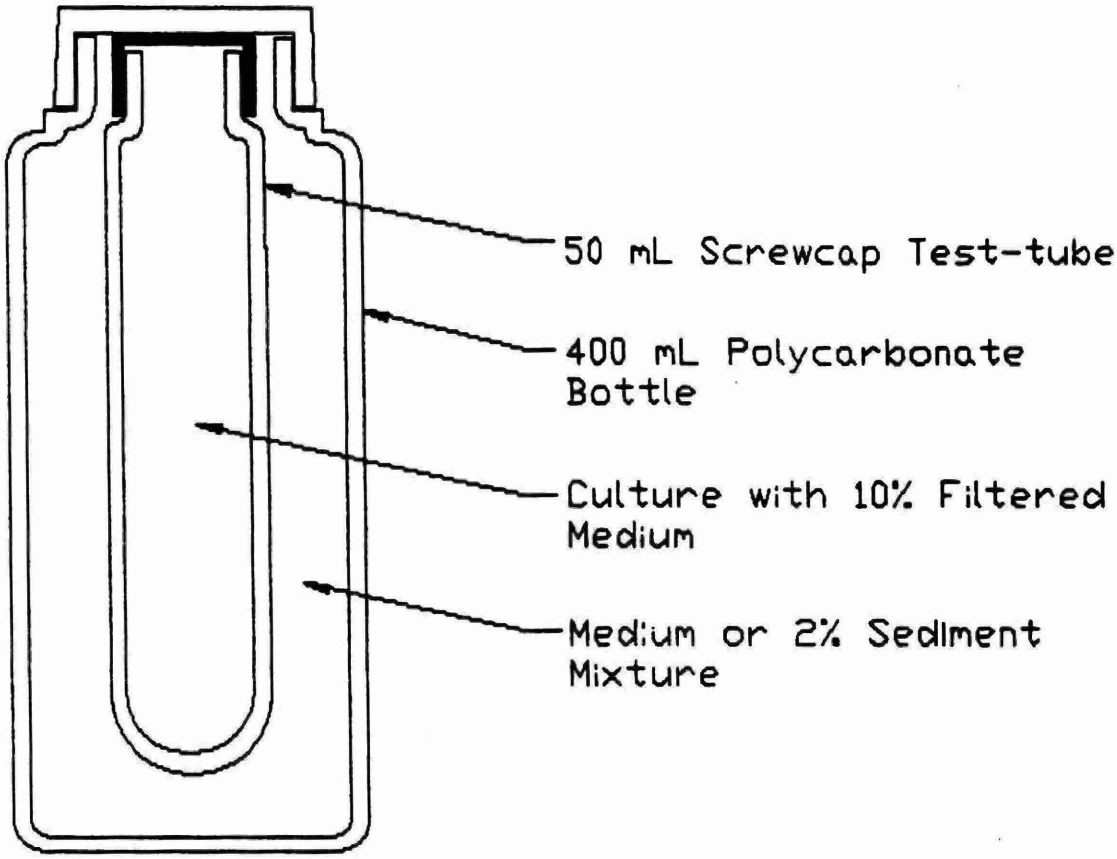
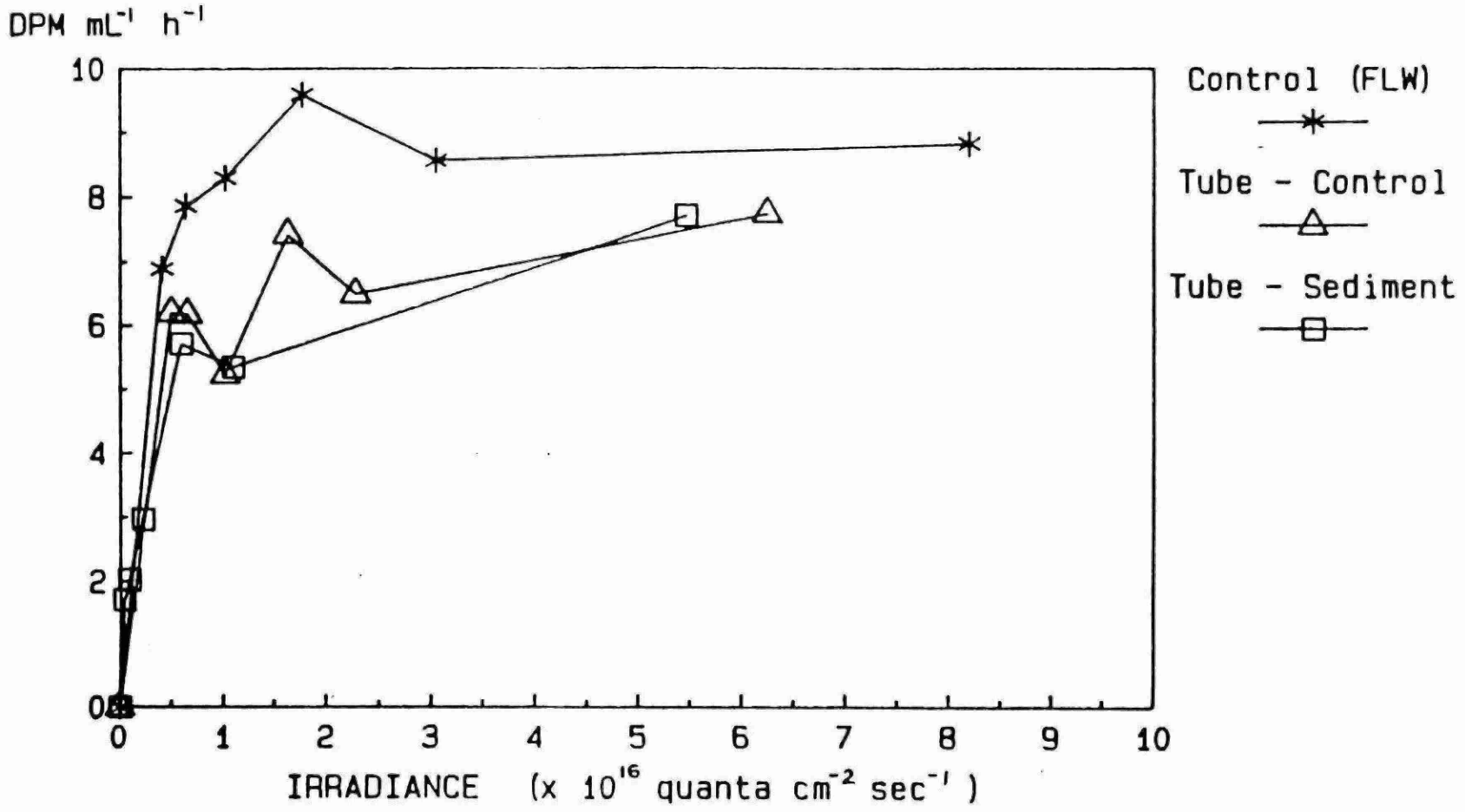

50 mL Screwcap Test-tube
400 mL Polycarbonate Bottle
Culture with 10% Filtered Medium
Medium or 2% Sediment Mixture

Fig 5



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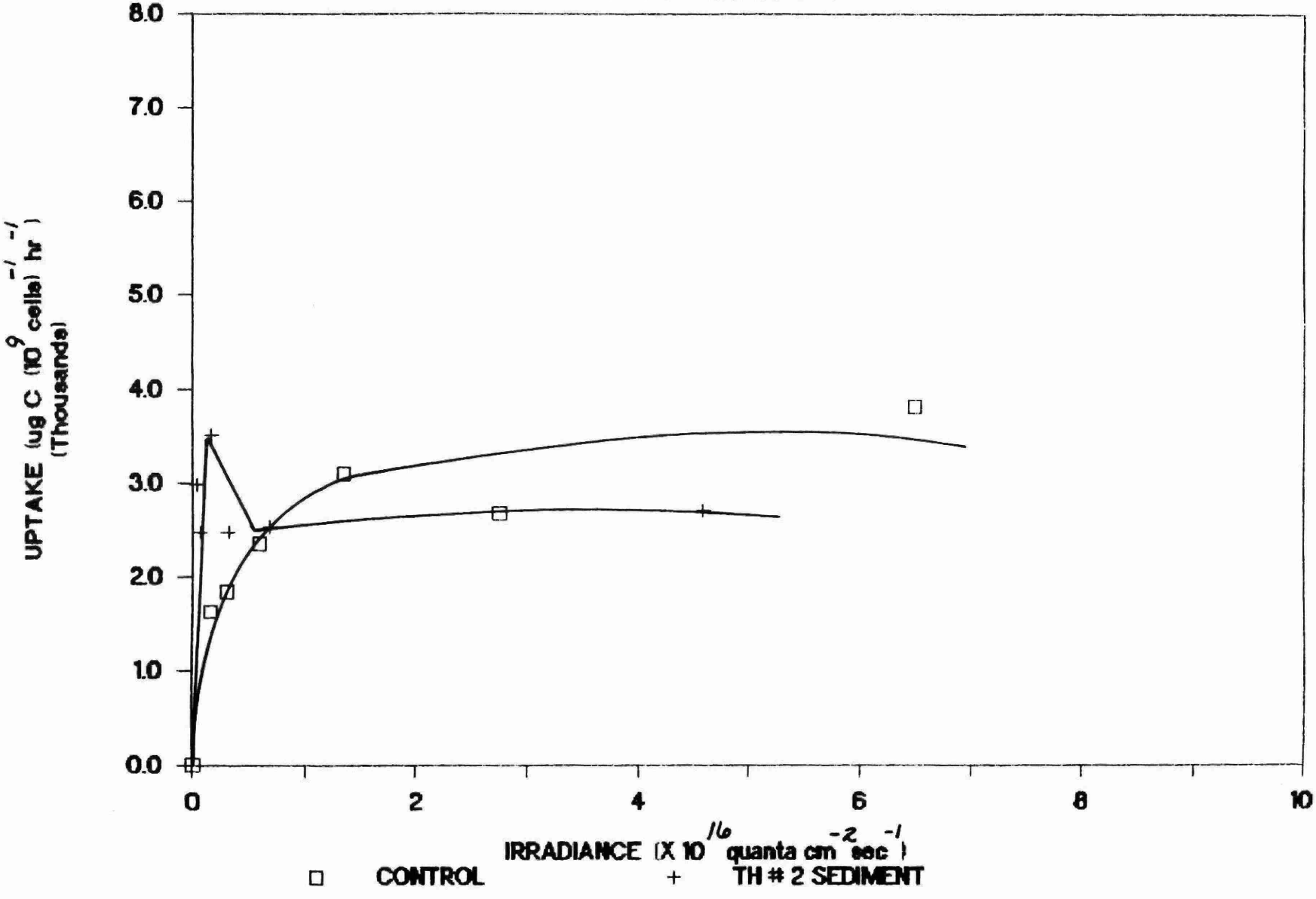
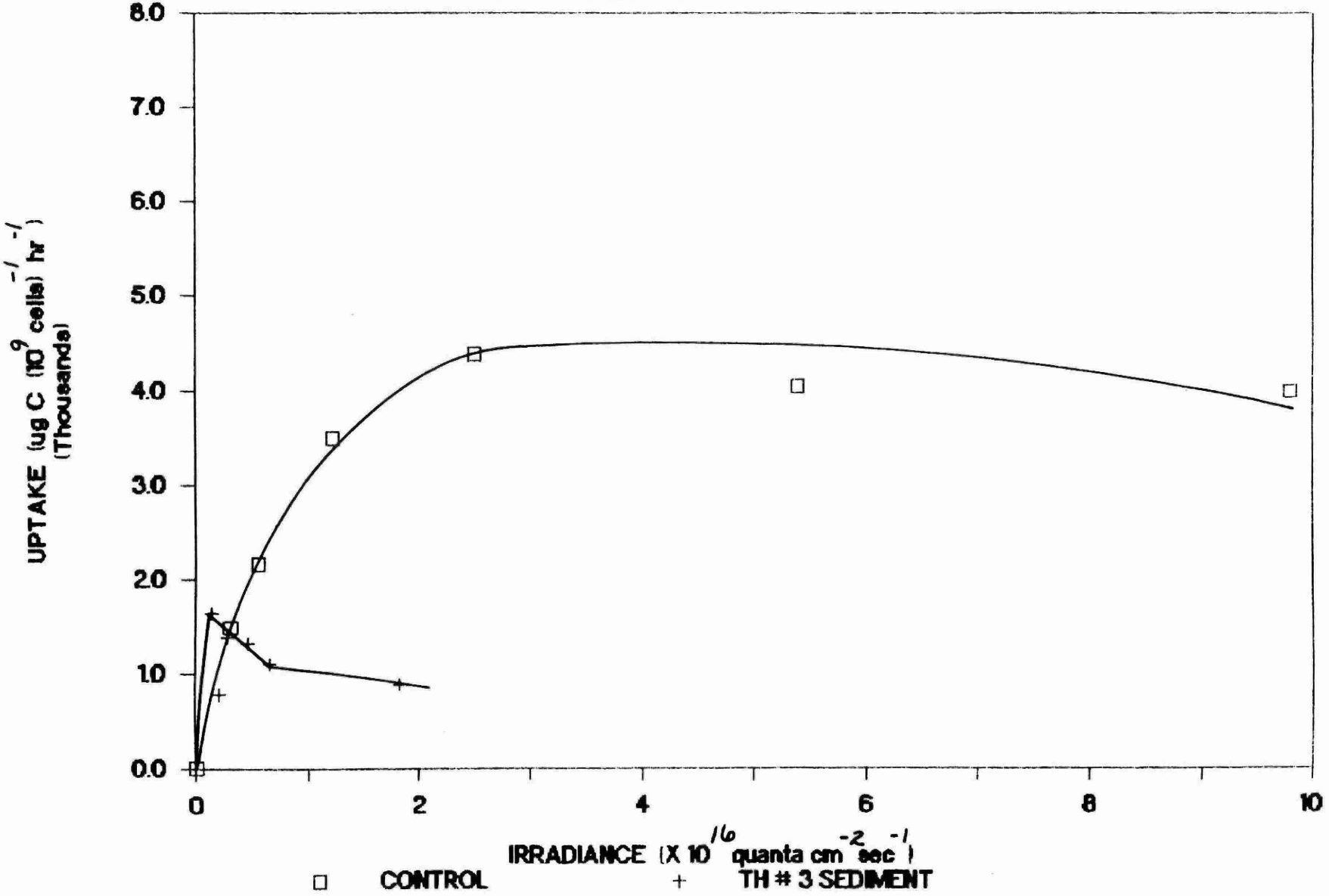
TORONTO HARBOUR # 2 - PHYTOPLANKTON

PHOTOSYNTHESIS - IRRADIANCE CURVE



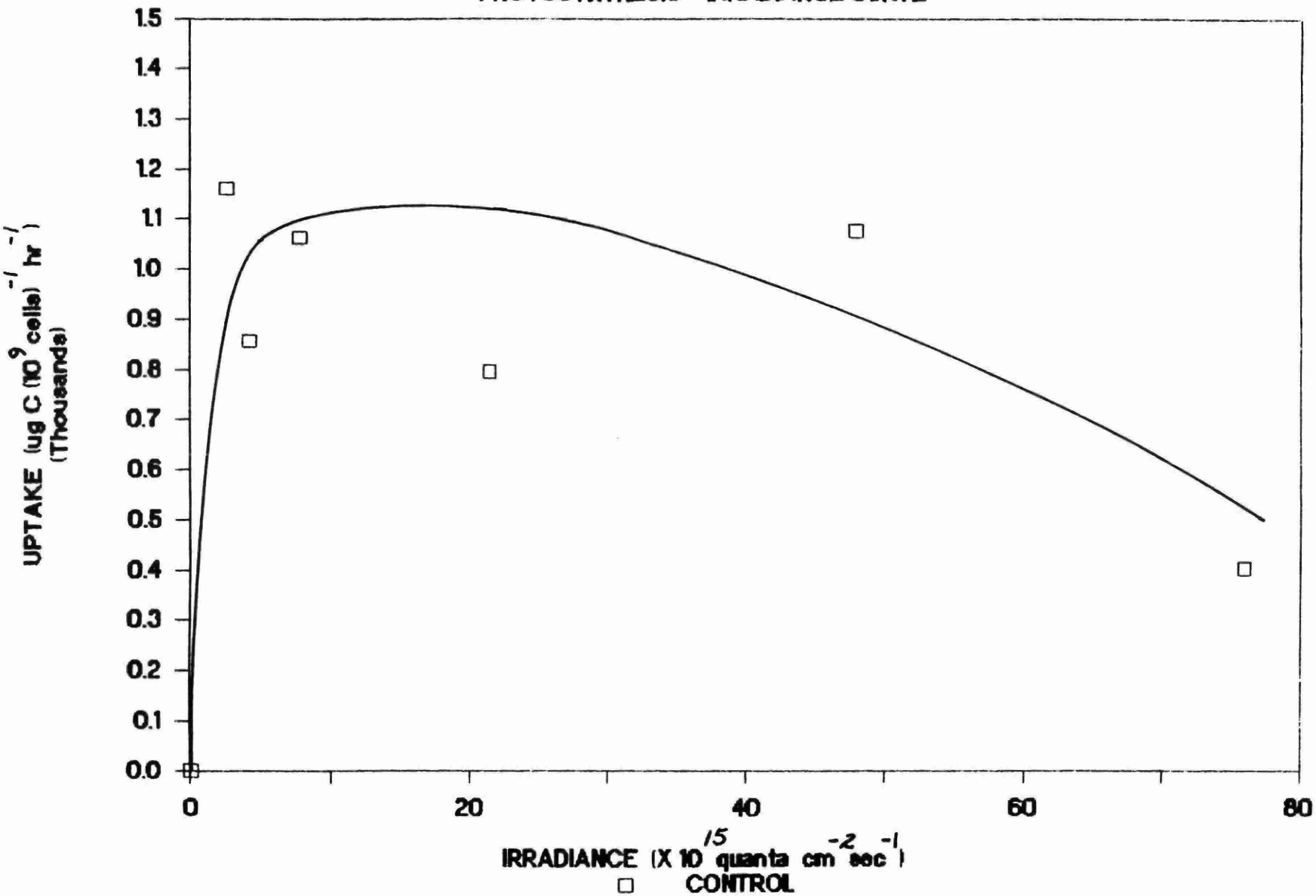
TORONTO HARBOUR # 3 - PHYTOPLANKTON

PHOTOSYNTHESIS - IRRADIANCE CURVE



SEDIMENT BIOASSAY - ANABAENA P-SUFF

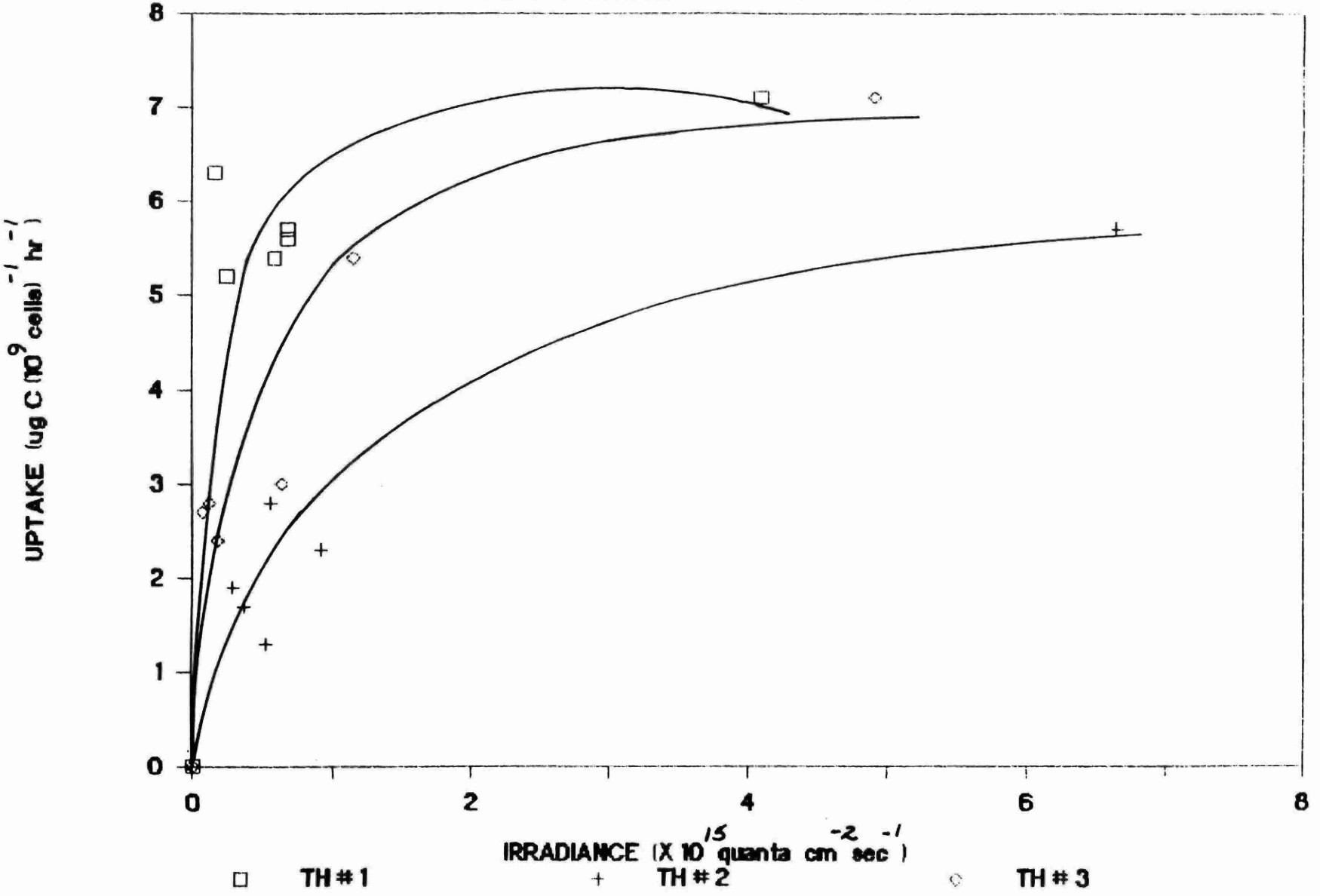
PHOTOSYNTHESIS - IRRADIANCE CURVE



ng °D

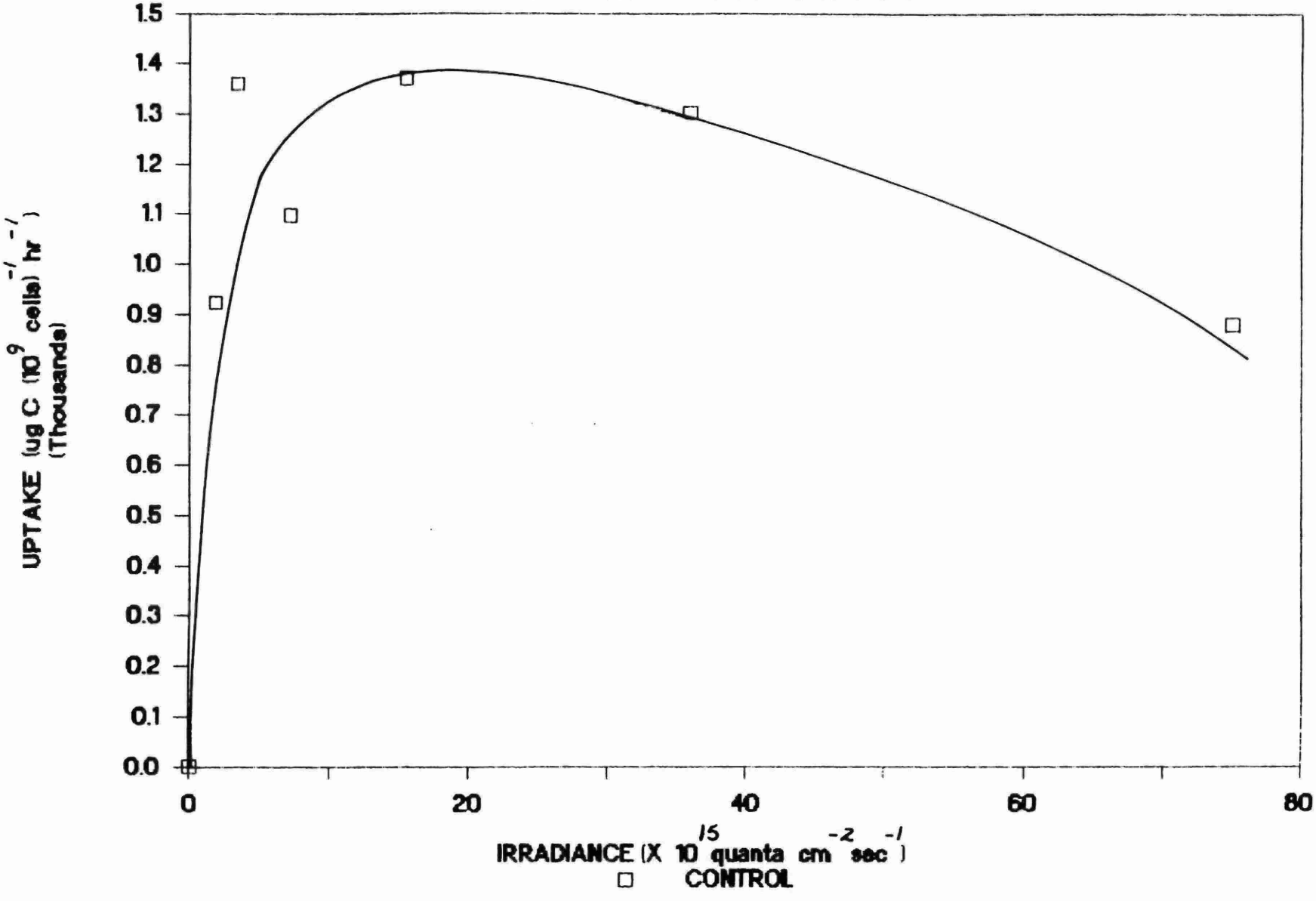
SEDIMENT BIOASSAY - ANABAENA P-SUFF

PHOTOSYNTHESIS - IRRADIANCE CURVE



SEDIMENT BIOASSAY - ANABAENA P-DEF

PHOTOSYNTHESIS - IRRADIANCE CURVE



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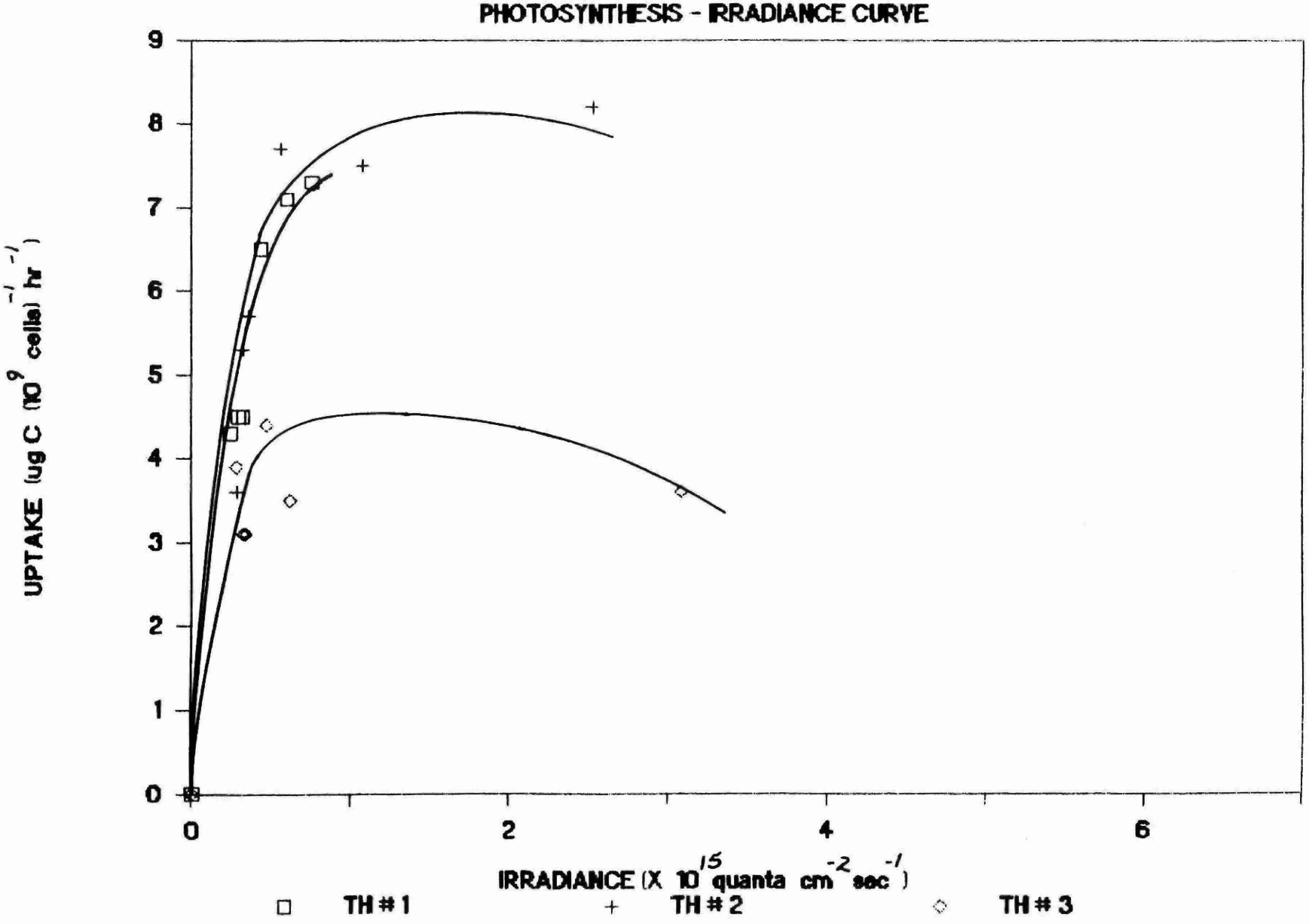
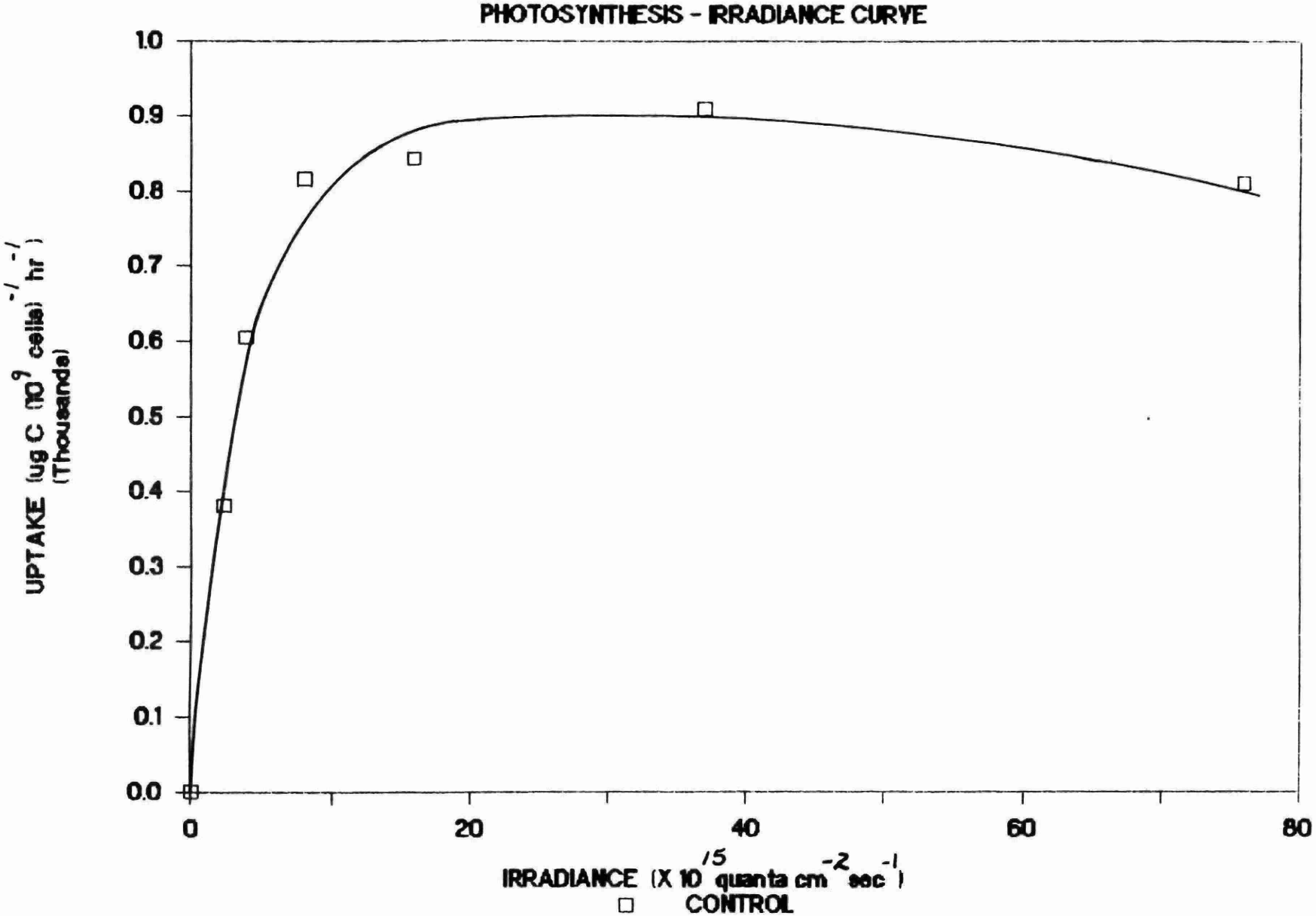


SEDIMENT BIOASSAY-ANKISTRODESMUS P-SUFF



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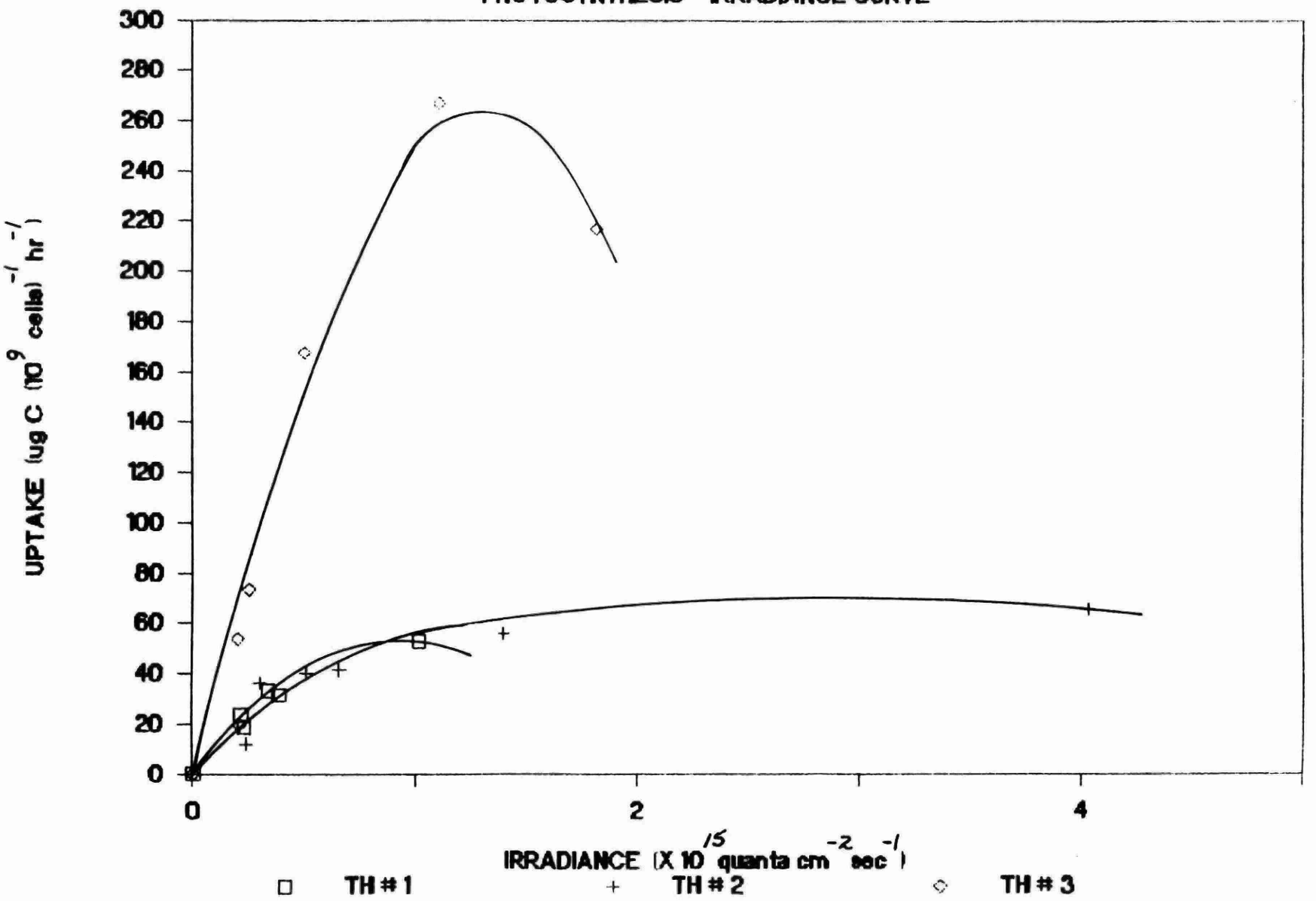
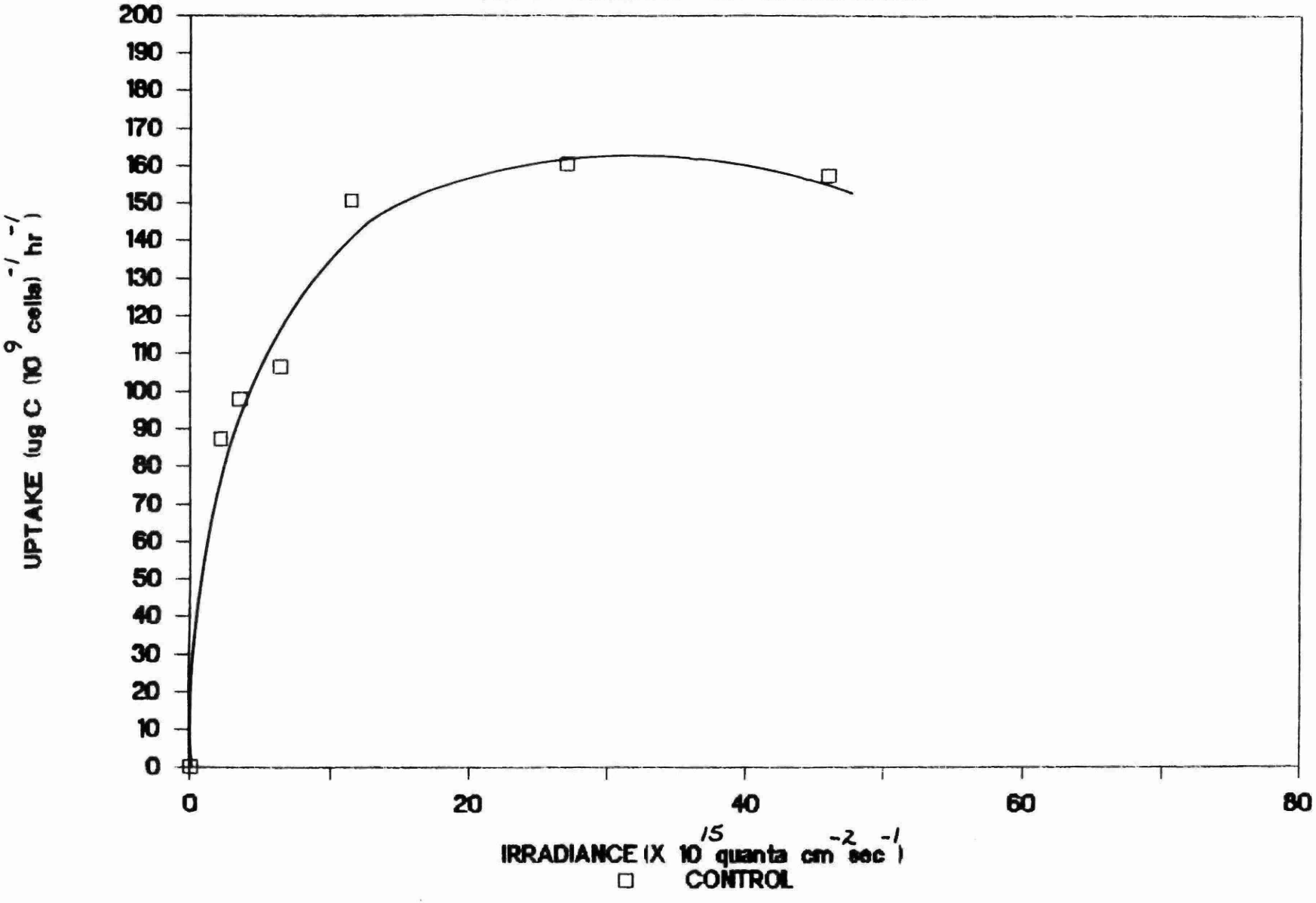


SEDIMENT BIOASSAY-ANKISTRODESMUS P-DEF



SEDIMENT BIOASSAY-ANKISTRODESMUS P-DEF

PHOTOSYNTHESIS - IRRADIANCE CURVE

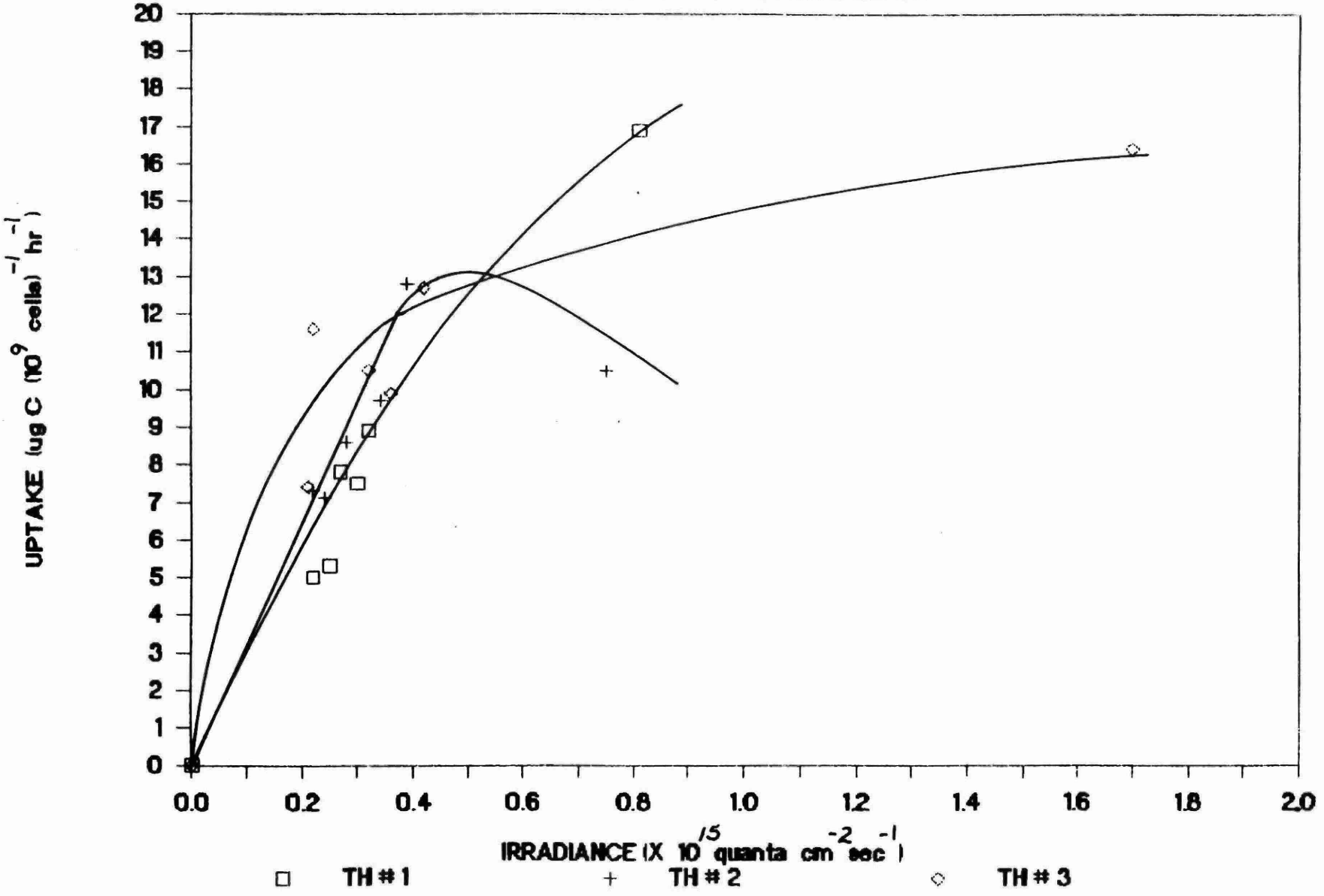
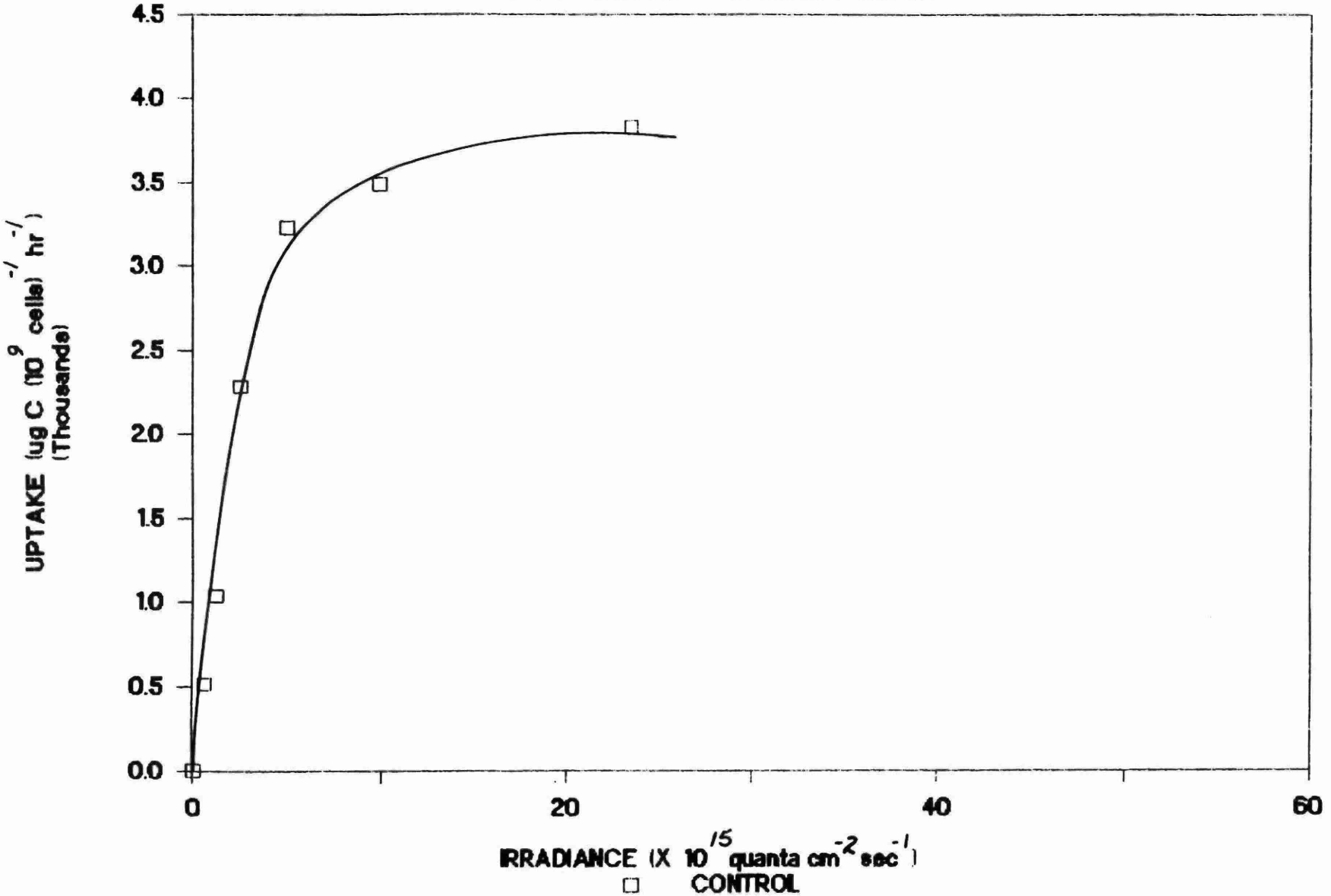


Fig 1.4.4

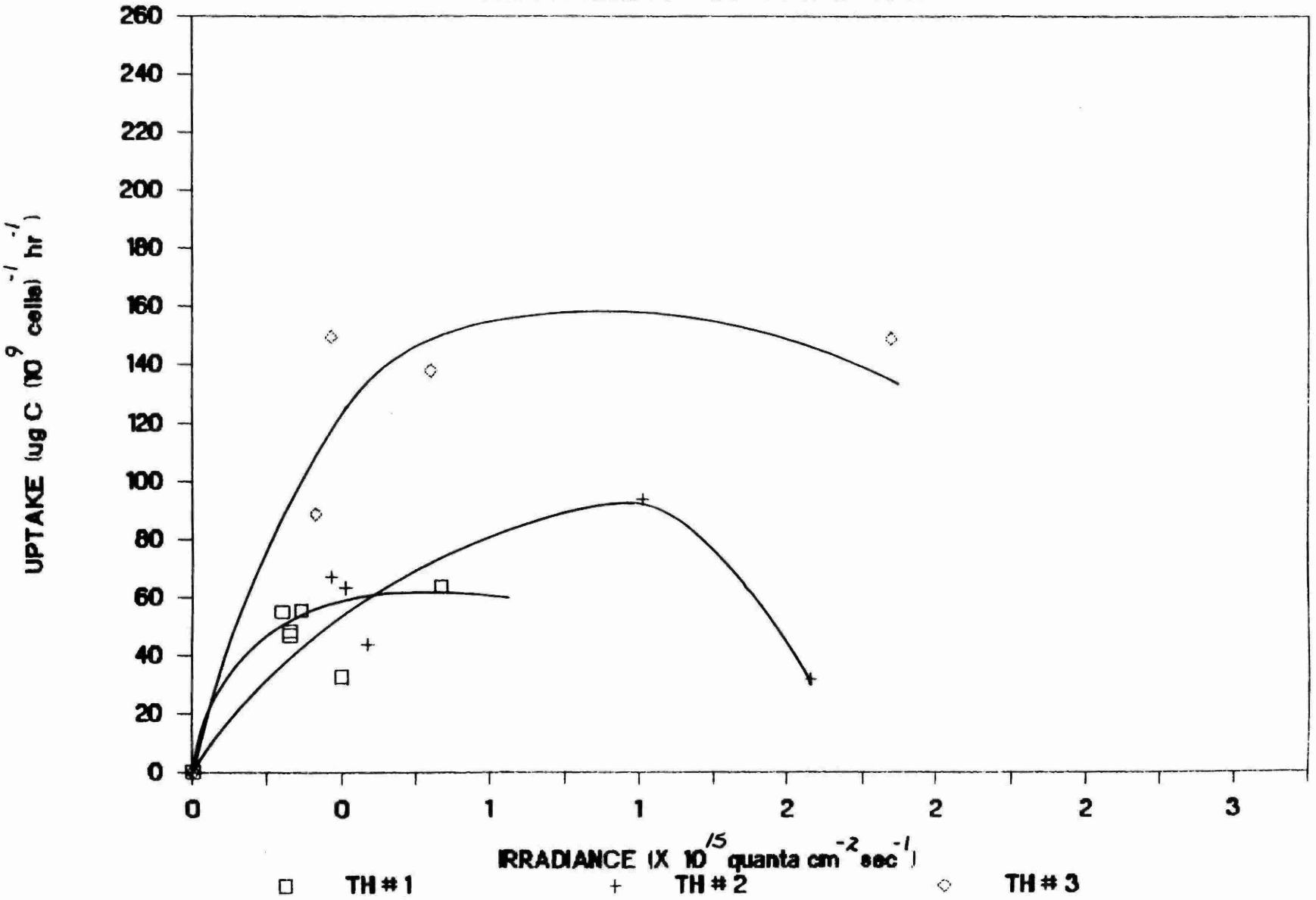
SEDIMENT BIOASSAY - DIATOMA P-SUFF

PHOTOSYNTHESIS - IRRADIANCE CURVE



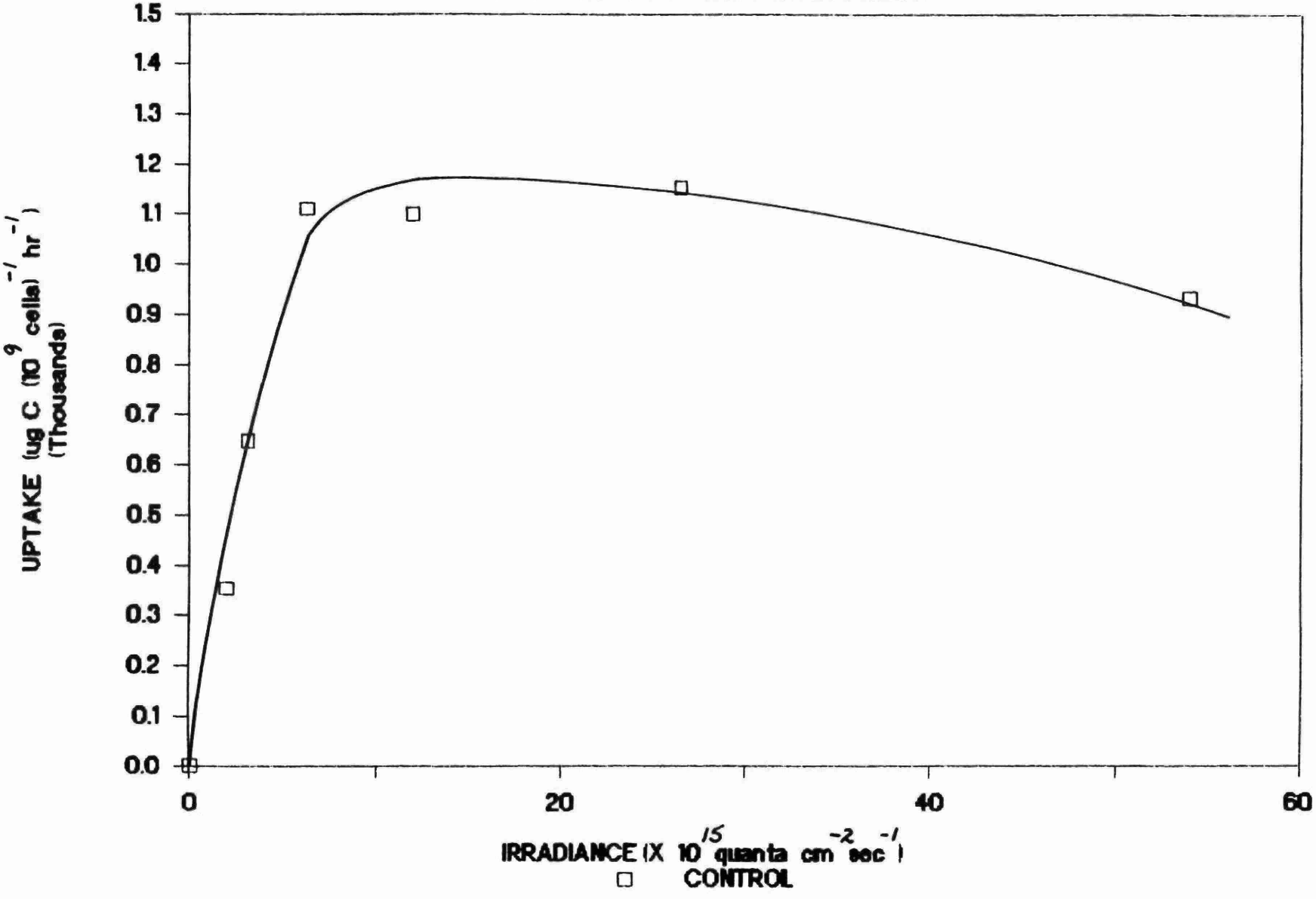
SEDIMENT BIOASSAY - DIATOMA P-SUFF

PHOTOSYNTHESIS - IRRADIANCE CURVE



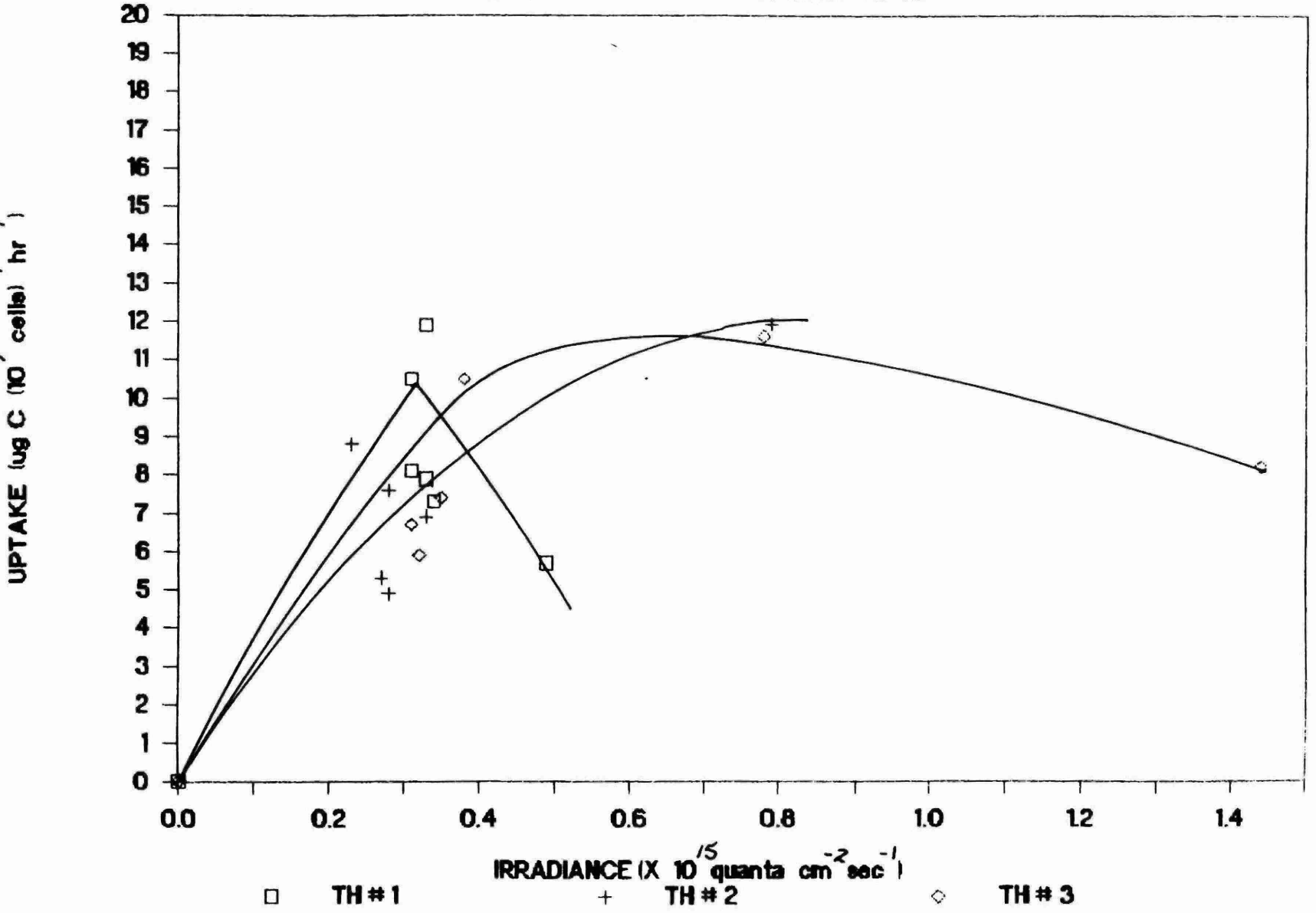
SEDIMENT BIOASSAY - DIATOMA P-DEF

PHOTOSYNTHESIS - IRRADIANCE CURVE



SEDIMENT BIOASSAY - DIATOMA P-DEF

PHOTOSYNTHESIS - IRRADIANCE CURVE



Appendix I

SUMMARY OF PHYTOPLANKTON IN OFFSHORE LAKEWATER, (LAKE ONTARIO) AND IN BIOASSAYS

SAMPLE: Toronto Harbour # 1 DATE: 12/06/80

ALGAL TAXON	UNITS at magnification 140 X	ENUMERATED 280 X	560 X	CELL CONC. (units/mL in original sample)	BIOVOLUME (μm^3)	ESTIMATED BIOMASS ($10^3 \mu m^3$/mL in original sample)
CYANOPHYCEAE						
Unidentified spp.			46	141.22	17.2	0.00
CHLOROPHYCEAE						
Closterium sp.	3			0.30	11242.3	0.00
Mougeotia sp.	8			0.80	720.2	3.37
Unidentified spp.			15	46.05	68.6	0.58
CHRYSOPHYCEAE						
Chromulina spp.			6	18.42	35.3	0.00
Kephyrion spp.			3	9.21	38.9	0.65
Ochromonas spp.			9	27.63	31.2	0.36
Unidentified spp.			12	36.84	41.9	0.86
BACILLARIOPHYCEAE						
Amphipleura sp.	1			0.10	4940	0.00
Asterionella formosa	614			61.40	639.6	0.49
Cymbella spp.	29			2.90	503.2	39.27
Fragilaria crotonensis	370			37.00	598.8	1.46
Fragilaria spp.	1019			101.90	842.6	22.16
Gomphonema sp.	2			0.20	746.9	85.86
Melosira granulata	94			9.40	5043	0.15
Melosira sp.	119			11.90	2912.1	47.40
Navicula spp.	7			0.70	564.6	34.65
Nitzschia spp.	22			2.20	416.1	0.40
Stephanodiscus sp.	2			0.20	1563.9	0.92
Synedra spp.	5			0.50	122.4	0.31
Tabellaria fenestrata	149			14.90	5637.2	0.06
Unidentified spp.			5	15.35	192.8	83.99
CRYPTOPHYCEAE						
Cryptomonas erosa			8	24.56	1551.8	0.00
Cryptomonas ovata	112			11.20	3610.8	38.11
Rhodomonas lacustris			5	15.35	284.1	40.44
Rhodomonas minutus			14	42.98	107.7	4.36
DINOPHYCEAE						
Glenodinium sp.	17			1.70	2066.6	0.00
Peridinium inconspicuum	9			0.90	2913	3.51
TOTAL 29	2582		123	635.81		409.37
				(= 0.64 X 10^3 cells/mL)		(= 0.41 mg/L)

Volume of orginal sample: 250 mLs Volume settled: 100 mLs

SAMPLE: Toronto Harbour # 2 DATE: 18/11/86

ALGAL TAXON	UNITS enumerated at magnification 140 X	280 X	560 X	CELL CONC. (units/mL in original sample)	BIOVOLUME (μm^3)	ESTIMATED BIOMASS ($10^3 \mu m^3$/mL in original sample)
CYANOPHYCEAE						
Unidentified spp.			13	39.91	17.2	0.69
CHLOROPHYCEAE						
Ankistrodesmus falcatus		4		3.40	19.7	0.07
Chlamydomonas spp.			5	15.35	289.6	4.43
Chlorella spp.		5		5.10	137.27	0.70
Closterium sp.	4			0.20	11242.3	2.25
Cosmarium sp.	1			0.05	1936.6	0.10
Oocystis lacustris		9		7.65	1826.9	13.98
Scenedesmus quadricauda		12		10.20	42.91	0.44
Unidentified spp.			27	82.89	68.6	5.69
CHRYSOPHYCEAE						
Chromulina spp.			10	30.70	35.3	1.08
Dinobryon sertularia		1		0.85	290.2	0.25
Ochromonas spp.			4	12.28	31.2	0.38
Unidentified spp.			17	52.19	41.9	2.19
BACILLARIOPHYCEAE						
Asterionella formosa	98			4.90	639.6	3.13
Cyclotella bodanica	13			0.65	4984.5	3.24
Cyclotella comta	11			0.55	1563.9	0.86
Fragilaria crotonensis	819			40.95	598.8	24.52
Melosira granulata	30			1.50	5043	7.56
Melosira sp.	66			3.30	2912.1	9.61
Navicula spp.		5		4.25	564.6	2.40
Nitzschia spp.	15			0.75	416.1	0.31
Synedra spp.	10			0.50	122.4	0.06
Tabellaria fenestrata	289			14.45	5637.2	81.46
Unidentified spp.			4	12.28	192.8	2.37
CRYPTOPHYCEAE						
Cryptomonas erosa		9		7.65	1551.8	11.87
Cryptomonas ovata	80			4.00	3610.8	14.44
Rhodomonas lacustris			6	18.42	284.1	5.23
Rhodomonas minutus			17	52.19	107.7	5.62
DINOPHYCEAE						
Gymnodinium sp.	4			0.20	16009	3.20
Peridinium inconspicuum	1			0.05	2913	0.15
TOTAL 30	1441	45	103	427.36		206.89
				(= 0.43 X 10^3 cells/mL)		(= 0.21 ng/L

Volume of orginal sample: 250 mLs Volume settled: 100 mLs

SAMPLE: Toronto Harbour # 3 DATE: 24/07 87

ALGAL TAXON	UNITS enumerated at magnification 140 X	280 X	560 X	CELL CONC. (units/mL in original sample)	BIOVOLUME (μm^3)	ESTIMATED BIOMASS ($10^3 \mu m^3$/mL in original sample
CYANOPHYCEAE						
Anabaena spiroides	1034			51.70	24.7	1.28
Unidentified spp.			21	64.47	17.2	1.11
CHLOROPHYCEAE						
Chlorella spp.		17		18.70	137.29	2.57
Closterium sp.	10			0.50	11242.3	5.62
Cosmarium sp.	3			0.15	1936.6	0.29
Oocystis lacustris	28			1.40	1826.9	2.56
Scenedesmus quadricauda		8		8.80	42.91	0.38
Scenedesmus spp.	14			0.70	848.8	0.59
Staurastrum sp.	3			0.15	1563.9	0.23
Unidentified spp.			32	98.24	68.6	6.74
CHRYSOPHYCEAE						
Chromulina spp.			39	89.03	35.3	3.14
Dinobryon sertularia		11		12.10	290.8	3.52
Kephyrion spp.			9	27.63	38.9	1.07
Ochromonas spp.			12	36.84	31.2	1.15
Unidentified spp.			31	95.17	41.9	3.99
BACILLARIOPHYCEAE						
Asterionella formosa	373			18.65	639.6	11.93
Cyclotella comta		5		5.50	1563.9	8.60
Fragilaria crotonensis	394			19.70	598.8	11.80
Navicula spp.		7		7.70	564.6	4.35
Nitzschia spp.		12		13.20	416.1	5.49
Synedra spp.			125	383.75	122.4	46.97
Tabellaria fenestrata	106			5.30	5637.2	29.88
Unidentified spp.			32	98.24	192.8	18.94
CRYPTOPHYCEAE						
Cryptomonas erosa		34		37.40	1551.8	58.04
Cryptomonas ovata		13		14.30	3610.8	51.63
Rhodomonas lacustris			31	95.17	284.1	27.04
Rhodomonas minutus			51	156.57	107.7	16.86
DINOPHYCEAE						
Ceratium hirundinella	3			0.15	41279.9	6.19
Gymnodinium sp.	5			0.25	2066.6	0.52
Peridinium inconspicuum	13			0.65	2913	1.89
TOTAL 30	1986	107	379	1362.11		334.37

(= 1.36×10^3 cells/mL) (= 0.33 mg/L)

Volume of orginal sample: 250 mLs Volume settled: 100 mLs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